The behavioural response of cattle (*Bos taurus*) to artificial weaning in two stages

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By

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ABSTRACT

Two factors presumed to affect the behavioural response of cattle (*Bos taurus*) to artificial weaning were investigated: the termination of nursing, and the physical separation of cows and calves. A two-stage process was used to disconnect these traditionally linked components. First, the behaviour of cows and calves was quantified in response to preventing nursing by having calves wear an antisucking device (Stage 1). Then the behavioural response of cows and calves to being separated was observed (Stage 2). Control pairs were weaned abruptly; nursing ended when cows and calves were separated. Preventing nursing while pairs were still together had almost no effect on measures of general activity with the exception of causing a slight increase in the rate of vocalizing. Calves wearing antisucking devices spent the same amount of time eating as controls. The behavioural responses of two-stage pairs to separation were favourably reduced compared to controls. In one study, two-stage cows vocalized 84% less than controls, spent 60% less time walking, and 13% more time lying, compared to controls. Two-stage calves called 97% less than controls, spent 61% less time walking, and 30% more time eating. In another study, preventing nursing for longer (3 versus 14 d) had no noticeable beneficial effects on the behaviour response to separation. In three separate trials two-stage calves gained more weight during the first week after separation from their dams. The two-stage process further reduced the behaviour responses when compared to weaning by fenceline contact. The benefits of two-stage weaning were also observed with dairy calves weaned from their dams at 5 weeks of age. The combined results of these studies indicate that the traditional method of weaning, by simultaneously terminating nursing and separating pairs, exacerbates the behavioural responses of cows and calves. Imposing these in two separate stages did not produce the same additive effect suggesting that the traditional weaning method produces a negative synergistic effect on the behaviour response. Based on the evidence two-stage weaning offers a viable production practice that is likely to improve the welfare of cows and calves.

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LIST OF ABBREVIATIONS & DEFINITIONS

ADG	Average Daily Gain
Artificial weaning	The imposed separation of dam and offspring by humans for the purpose of terminating nursing
d	Day
GEE	Generalized Estimating Equations
h	Hour
kg	Kilogram
km	Kilometre
LS	Least Squares
m	Metre
min	Minute
PROC GENMOD	Procedures of General Modelling
SAS	Statistical Analysis Software
SD	Standard Deviation
SE	Standard Error
Two-stage weaning	The termination of nursing for a period while the dam and offspring are still together (Stage 1), followed days later by their physical separation (Stage 2).

1.0 GENERAL INTRODUCTON

The traditional management practice of artificial weaning livestock has been incorporated to improve production efficiency by maximizing the reproductive potential of the dam, by allowing for the marketing or specialized feeding of the offspring (e.g., Myers et al., 1999) and, in certain species, by maximizing the quantity of milk available to humans. However, because weaning is artificially imposed, several issues of concern have arisen.

Artificial weaning is a distressing experience for dams and their offspring. Overt behavioural responses caused by weaning last for several days. Changes in the behaviour of cows and calves is remarkable enough that any casual observer would easily conclude the animals were in a state of increased anxiety.

Artificial weaning represents a fundamental animal welfare problem not only from the perspective of public perception but also by most scientific definitions. The behavioural response of dams and their offspring would suggest a negative emotional state during the days that follow weaning and it has been suggested that affective states or feelings are an essential element of assessing animal welfare (Duncan, 1993). The fact that weaning is imposed artificially counters the natural species-specific process and the principle of '*telos*' responsible for the persistent relationship between the dam and offspring (Rollin, 1995). As the stress associated with weaning has been linked with an increased incidence of disease (e.g., calves: Harland et al., 1991) it would appear that imposing weaning also fits the basic criteria of a welfare concern suggested by Moberg (1993) as anything that puts animals at increased susceptibility to disease, signs of impaired growth, etc.

A vast majority of the research in this subject area to date has taken an extremely practical and applied approach to resolving the major problems associated with weaning distress, with seemingly less regard for exploring the fundamental behavioural mechanisms that underlie the problems.

1.1 Thesis objectives

The primary objective of this thesis was to study the basic components of artificial weaning. Specifically, I set out to isolate the effects of 1) terminating nursing, from 2) the effects of separating cows and calves, to better understand how each of these factors affect the response of beef cattle to weaning.

Chapter 2 presents a review of the available literature on weaning from a variety of farm animal species but with an emphasis on what is known about this practice with respect to cattle. The chapter briefly presents some of the ecological approaches to understanding the process of weaning in its natural context as well as what is known about the natural weaning process in farm animals. The main focus of Chapter 2 is an examination of various methods for weaning farm animals that have been investigated, the rationale for these approaches and some discussion of what these studies have taught us about the practice of artificial weaning and what can be done to resolve the problems still associated with it.

Chapter 3 presents the original experimental model and design that I created to isolate the effects of preventing nursing from the effects of separating cows and calves. The results of those trials redirected the research focus of this thesis by exploring the possible use of the experimental model and two-stage protocol as an alternative method for weaning cattle. The next step examined how the duration of preventing nursing affected the response of cows and calves to subsequent separation (chapter 4). The chapter that follows (chapter 5) combines a series of four separate experiments more focused on assessing the practical on-farm implications of applying a two-stage weaning procedure. These experiments further examined effects of the duration of preventing nursing on behaviour and examined the effects of this weaning strategy on the weight gain of calves. These trials also collected further information about the consequences of preventing pairs from nursing, while otherwise allowing them full contact and social interaction, by examining the spatial relationship between cows and calves under those circumstances. In the sixth chapter the proposed method of weaning cattle in two-stages is compared to the most popular alternative method of weaning, separating cows and calves with fenceline contact. In chapter 6, I return to investigating more fundamental aspects of the weaning process in cattle by studying whether the beneficial effects of preventing nursing in the presence of the dam are affected by the offspring's level of dependence on the dam.

The experiments presented in this thesis are the first to isolate and examine the effects of preventing nursing apart from the effects of physically separating the dam and offspring when weaning is imposed. The results will offer new insight into how these two factors contribute to the overall response of cattle to weaning while other studies in this thesis will offer a practical solution to reducing the stress of artificial weaning. It is hoped these results will stimulate other scientists to further investigate and understand the nature of the relationship that exists between dams and their offspring and how their relationship is affected by livestock production practices.

2.0 REVIEW OF THE LITERATURE

2.1 Abstract

The artificial weaning of farmed animals provokes very striking behavioural responses by dams and their offspring, most notably increased vocalizing and general activity. These responses are sustained for a period indicative of prolonged distress and poor welfare. Another issue of concern is that following artificial weaning the offspring of many species can fail to thrive and are at increased risk of disease susceptibility.

The behavioural responses to artificial weaning are not merely artifacts, but rather can be viewed as natural responses to factors associated with the termination of nursing and the physical separation of the dam and offspring. Our understanding of these responses has been enhanced by concepts from behavioural ecology such as the theories of "parent-offspring conflict" (Trivers, 1974) and "honest signaling of need" (Zahavi, 1987).

Insights gained from research exploring various possible alternative weaning methods, points to a logical area for further investigation which could help us to further understand the responses to abrupt weaning. This review considers where there may be room for further investigations to improve artificial weaning methods.

2.2. Introduction

As a theoretical concept weaning has often been investigated for its relation to hypotheses about parental investment. It has even been suggested the definition of weaning should encompass changes in all the various ways that dams (mammalian or even avian) provide care to their offspring (Martin, 1984). However, for the purpose of this review and the studies that follow, weaning will refer to the termination of suckling (Martin, 1985).

Natural weaning involves not only transition of the offspring to full nutritional independence, but also increased social independence from the parents. These changes are understood to occur gradually (Lee, 1997). The definitive point of natural weaning in farmed animals is largely unknown but it is reported to vary, even within species (see Gonyou and Stookey, 1987).

In practice, farmed animals are weaned artificially and abruptly. Most often the dam and offspring are forcibly weaned by physical separation, which is quite different from the natural course. Reasons for artificially weaning farm animals include maximizing the subsequent reproductive efficiency of the dam and allowing for the marketing or specialized feeding of the offspring (e.g., Myers et al., 1999). In some farmed animals (e.g., goats, sheep, cattle), early weaning is imposed to maximize the quantity of dam's milk available to humans. Studies examining the nitrogen isotope levels of cattle teeth from archeological finds show evidence that artificial weaning may have been imposed as early as *c*. 4000 BC (Balasse and Tresset, 2002).

Under modern systems of livestock production it is not uncommon for the offspring to be subjected to an array of other procedures that may compound their stress around weaning time. Depending on the species these can include being regrouped with unfamiliar animals, moved to new paddocks, provided different food, and possibly transported. Species such as cattle may undergo further processing procedures such as vaccination, dehorning and castration.

In most species, remarkable behaviour changes occur following artificial weaning such as increased vocalizing and walking. This review will provide evidence about the magnitude and extent of the changes caused by artificial weaning.

An evaluation of specific behavioural responses using theories from behavioural ecology (parent-offspring conflict and honest signaling) has helped to give context and insight into the biological basis of certain responses. This review analyzes alternative weaning strategies that have been previously explored to mitigate the distress caused by artificial weaning. In addition this review raises questions that remain unanswered about the factors that affect the behavioural response of dams and their offspring to artificial weaning.

2.3 Natural weaning

Information about natural weaning in farmed animals is limited (cattle: Reinhardt and Reinhardt, 1981; pigs: Jensen, 1986; sheep: Arnold et al., 1979). Documenting the natural process accurately and completely is difficult and requires very extended and intensive observations, which may explain the lack of information in this area. However, the studies that do exist inevitably depict the changes toward nutritional and maternal independence, as occurring gradually, even over several months, in a range of species (Lee, 1997).

It seems logical to assume that behaviour changes associated with natural weaning would mirror changes in the dam's milk production, which declines gradually after reaching its peak. The ontogeny of suckling behaviour over extended periods (e.g., changes in the frequency and duration of nursing) has been described for many species and has often been used to gauge parental investment. However, Cameron (1998) concluded these characteristics alone correlate poorly with milk transfer and so may not offer much insight into the timing or progression of natural weaning. Milk yield measured directly though, has been established as playing some role in the weaning process in sheep (Arnold et al., 1979). Ewes that produce less milk wean their young earlier and well-fed ewes were found to naturally wean their lambs later than poorly fed ewes.

A unique perspective on the weaning process is offered by the parent-offspring conflict theory, which alleges the dam and offspring will be in disagreement over the allocation of resources and the timing of natural weaning (Trivers, 1974). Beyond a certain level of maternal investment or age of the offspring, it is theorized that the respective interests of dams and their offspring diverge or conflict. The dam may, at a certain point in time, benefit more by investing resources in future offspring and thus reduce or terminate her investment in the present offspring (e.g., by weaning). On the other hand, the offspring would likely benefit from any further investment the dam is willing to give and should work to preserve access to resources and seek to delay weaning.

It is natural to assume that the dam, as the primary holder of resources (e.g., milk, ability to protect the young) is more likely to control the timing of weaning. Indeed, under semi-natural conditions, sows progressively reduce their nursing frequency and increase the time that they spend away from their piglets (Jensen and Recén, 1989). As lactation progresses dams are also reported to initiate fewer suckling bouts and more frequently terminate them, both in cattle (Reinhardt and Reinhardt, 1981) and in pigs (Jensen, 1986).

In one of the only studies reporting on natural weaning in cattle, Reinhardt and Reinhardt (1981) observed that the cessation of nursing in zebu (*Bos indicus*) occurred when calves were between 7 and 14 months of age. The authors reported the changes in behaviour that resulted in weaning actually occurred over a distinct two-week period. They also observed that calves were prevented from nursing by their dams, suggesting some degree of conflict and providing support for Trivers' theory of parent-offspring conflict over weaning. Reinhardt and Reinhardt (1981) also observed that female calves were weaned earlier than males (on average 8.8 vs. 11.2 months of age), which supports the notion that dams may differentially invest according to sex of the offspring.

There is no easy way to know how conflict that might arise during the process of natural weaning would compare to the stress imposed by artificial weaning. It seems reasonable to assume that under natural conditions the normal weaning process would have evolved toward an arrangement that did not impart unfavorable levels of stress on either the dam or the newly independent young.

2.4 Behavioural responses to artificial weaning

The most common method used to wean farmed animals and to terminate suckling is to physically and permanently separate the dam and the offspring. The behavioural responses to this artifical and abrupt weaning practice are similar among a wide range of species and the basic behaviour patterns provoked are essentially the same for dams and offspring. The two most obvious responses are an increase in vocalizing and an increase in time spent walking or general movement if physical space is limited (cattle: Weary and Chua, 2000; Flowers and Weary, 2003; Price et al., 2003; horses: McCall et al., 1985; Heleski et al., 2002; lambs: Orgeur, 1998, Orgeur et al., 1999; pigs: Weary and Fraser, 1997; wapiti / red deer: Haigh et al., 1997; Church and Hudson, 1999; Pollard and Littlejohn, 2000).

Observations of beef cattle on extensive range pastures by Watts (2001) found that vocalizing and walking were inversely related to the time required for cows and calves to reunite following brief separations that occurred naturally on a daily basis. These behaviour changes may well represent the most truly functional response of the dam and offspring to separation. From a theoretical perspective, certain distress behavioural responses to artifical weaning by separation, for example vocalizing, may also serve as an indicator of an individual offspring's need for maternal care or resources (Weary and Fraser, 1995). Piglets that suckle the anterior teats of the sow and receive more milk would suffer more nutritional deprivation after weaning and they also perform more low-frequency "begging calls" after weaning (Mason et al., 2003). Interestingly, dams have been reported to be more vocal than their offspring after weaning (sheep: Orgeur et al., 1999), which may be logical from a theoretical perspective regarding their level of investment in the offspring that has suddenly gone missing.

In addition to the increase in vocalizing and walking, it is common that both the dam and offspring spend less time eating in the few days after weaning is imposed (cattle: Stookey et al., 1997, Price et al., 2003; horses: Houpt et al., 1984). This has in fact been shown to result in reduced feed intake, reduced weight gain and even weight loss, in various species (cattle: Price et al., 2003; Weary et al., 1999; horses: Houpt et al., 1984). This consequence is of particular importance because sustained weight gain by the young is considered an important aspect of efficient livestock production.

Another change observed after weaning is in aggressive behaviour. Offspring in particular may show higher levels of aggression after weaning (cattle: Veissier and le Neindre, 1989; pigs: Yuan et al., 2004; horses: McCall et al., 1985). This has been described as an indicator of frustration resulting from maternal separation or possibly the thwarting of nursing, or both. The aggression may arise in part from competition food or other resources or be due to the common practice of mixing unfamiliar animals at the time of weaning. This is done to facilitate feeding and management of large groups. Furthermore, increased general activity after weaning may increase the overall frequency of encounters, agonistic or otherwise.

Other aberrant behaviour patterns can occur after weaning in some species including the performance of belly-nosing by piglets especially those weaned at a very young age (Weary et al., 1999) and the performance of nonnutritive sucking by foals (McCall et al., 1985). Weaning stress has also been linked to the development of oral stereotypies in horses later on in life (Nicol et al., 2005). These peculiar behaviour patterns are generally oral in nature and would appear to be closely related to feeding. This might explain why similar aberrant behaviour patterns have not been reported for dams following weaning.

Weaning is also associated with physiological changes indicative of distress. Peripheral catecholamine concentrations in calves and epinephrine concentrations in their dams have been shown to increase following separation and subsequently show a significant decrease when the cows and calves are reunited (Lefcourt and Elsasser, 1995). In hroses, weaning has been shown to increase plasma or serum cortisol levels (McCall et al., 1987; Malinowski, 1990) salivary cortisol and heart rate (Moons et al., 2005).

2.4.1 Behavioural response characteristics

The latency to initiate behavioural responses following weaning varies between species and with the age of the offspring. Generally for foals (McCall et al., 1985) and piglets (Weary et al., 1999) the response is reported to be almost immediate. Ralls et al. (1986) classify horses as a 'follower' species and thus their short response latency may be indistinguishable from the response they show to short-term separations. Under natural conditions young piglets spend most of their time with the sow and thus a similar explanation might also apply in their case.

By comparison the behavioural response of cattle to weaning generally has a longer latency that can be affected by their age, especially for very young calves (Weary and Chua, 2000; Flowers and Weary 2003). Ralls et al. (1986) have classified cattle as a 'hider' species, meaning that young calves may sometimes be left behind by their dams, lying in waiting, while the cow is away foraging. Thus, from an early age cattle pairs may be accustomed to reasonably prolonged separations, thus delaying their behavioural responses at the time of forced weaning until the time period of accustomed separation has elapsed.

The intensity or magnitude of changes in behaviour following artificial weaning is quite striking. For example, Price et al. (2003) noted that vocalizing by beef calves over the first 3 days following artifical weaning was 2000 to 4000 times greater than for unweaned controls. In the same experiments, weaned calves spent 28.1% of their time walking while unweaned calves spent only 8.6% of their time walking. Eating by controls constituted 41.1% of observed time while calves weaned in the traditional manner of abrupt separation spent 23.7% of their time eating.

Studies on horses have found that the day before weaning foals vocalized on average 0.1 times / h whereas after weaning the frequency was 37.4 / h (Moons et al., 2005). The same study observed that walking by foals increased by 65% over baseline levels.

Very young foals (Houpt et al., 1983) and calves (Weary and Chua, 2000) have been found to remain relatively inactive when separated from their dam during the first week of life compared to separations performed during subsequent weeks. The behavioural response of calves to total separation from the dam is reduced when it occurs before 24 h of age but the response is already significantly greater for calves separated 4 d after birth (Weary and Chua, 2000). The response trend of cows follows a similar pattern in terms of a reduced response when separated from their very young calves (Weary and Chua, 2000; Flowers and Weary, 2003). The specific effects of weaning age on older calves (e.g., 10 months) have not been closely examined although hypotheses could be formulated and tested, as presumably older calves are receiving less milk from their dam and thus less dependent and closer to the final point of natural weaning.

Another important characteristic of the response to weaning is the persistence of those changes from normal behaviour, which can last for several days. This is interesting because few procedures or management practices imposed on farm animals result in such long-lasting changes in behaviour. A gradual decrease in the behavioural response a few days past weaning has been cited as evidence that the animal is adapting (Price et al., 2003). However, it is interesting that with prolonged transportation, for example, a decline in the physiological measures of stress over time has been interpreted as an exhaustion of the system (Schwartzkopf-Genswein et al., submitted) rather than adaptation, per se. By this logic a return to baseline behaviour levels 3-5 days after weaning may be due in part to exhaustion or the animal's inability to maintain their response levels of vocalizing and elevated activity. As an example, it is common that some animals vocalize to the point of being hoarse and so perhaps a decline in vocalizing 3-5 days after weaning may be due in part to suppression of vocalizing caused by irritation of the larynx as well as possibly their adaptation over time to having been weaned.

For cattle, the greatest differences in time budgets compared to baseline occur within 48 h after separation (Weary and Chua, 2000; Flowers and Weary, 2003; Price et al., 2003), which may suggest a peak in the motivation of animals to reunite (Price et al., 2003). After this point behaviour changes generally decline which is thought to suggest adaptation to being separated (Price et al., 2003). However, significant differences in behaviour compared to baseline levels have been reported to persist for up to 72 h after weaning (Veissier et al., 1989b). The adaptation after weaning has been shown to be similar for calves whether they are reared by their dam or by a foster mother (Veissier et al., 1989b).

2.4.2 Perspectives on the behavioural responses from behavioural ecology

Variation in behaviour is a natural phenomenon and not every animal responds identically to artificial weaning, not even full-sibling littermates weaned from the same sow at the very same point in time (Mason et al., 2003). These individual differences between offspring in their response to weaning have been the subject of some investigation (Mason et al., 2003). In swine production, narrow economic margins necessitate a more detailed understanding of how best to maximize growth to achieve balanced, uniform groups throughout the chain of production. Changes in weaning management practices, for example segregated early weaning, have created a need for research in this area to understand the responses of piglets to weaning and how best to mitigate any negative consequences.

Two theoretical perspectives from the basic biological literature have been incorporated into some applied ethological studies to try to expand our understanding of the behavioural responses to weaning. The two theories from behavioural ecology relate to 1) parent-offspring conflict around the time of weaning and 2) the behavioural responses of the offspring to weaning as possible signals of biological need. The focus, when incorporating behavioural ecology theories into applied ethology studies, is on understanding the behavioural responses not as aberrant reactions to artificial weaning but as having functional effects. In other words, to artificially impose weaning must cause behavioural responses that are at best amplified reflections of the natural responses that dam-offspring pairs would perform if such a separation were to occur in a natural setting.

2.4.2.1 Parent-offspring conflict theory

Parent-offspring conflict theory contends the dam and the offspring may be in disagreement over the level of parental investment provided, and thus the precise timing of natural weaning (Trivers, 1974). Up to a certain point in time both the dam and offspring benefit from maternal investments, especially when the offspring are young and fully dependent. Indeed the offspring will continue to derive benefit from maternal investments up to sexual maturity. However, the dam at some point in time would enhance her investment by conserving her resources and energy for future offspring. This, at least in theory, has the potential to give rise to conflict between the dam and offspring over the allocation of resources, particularly as the offspring becomes increasingly independent and able to survive on its own.

Predictions arising from parent-offspring conflict theory have been tested in weaning studies of sows and piglets using a “get-away” housing system (Pitts et al., 2002). This experimental set-up allows sows the opportunity to exercise more control of the frequency with which they nurse their young and the time they spend with them; a more natural situation than traditional farrowing systems. In such systems sows will gradually increase the amount of time they spend away from their litter as the piglets get older (Pitts et al., 2002). Sows also instigate fewer nursing bouts as time passes (Pitts et al., 2002). Piglet behaviour also appears to be aligned with predictions arising from parent-offspring conflict theory. Specifically, piglets increase the amount of time they spend massaging the sow's udder as they get older; a behaviour performed to initiate a nursing bout and gain access to resources (Pitts et al., 2002).

In traditional farrowing crates, sows gradually increase the time they spend lying ventrally with their teats concealed underneath them, preventing piglets from initiating nursing bouts. As lactation progresses, females of various species have been observed to reject nursing attempts by their offspring, providing some support for the theory of parent-offspring conflict (e.g., cattle: Reinhardt and Reinhardt, 1981; deer, Gauthier and Barrette, 1985; macaques: Rosenblum, 1971). These behaviour patterns, particularly by the dams, provide evidence that they are trying to reduce investment in their present offspring and wean their young, likely as an evolutionary strategy to help ensure success of future offspring.

2.4.2.2 Responses to weaning as honest signals of need

Signals are specific adaptations designed to modify the behaviour of the receiver and so not all responses to weaning (i.e., increased walking, decreased eating and lying) should be considered as signals between the sender and a receiver (Weary et al., submitted). However, the vocal behavioural response of the young to artificial weaning in particular can be evaluated as a form of signaling directed at the dam in an attempt to obtain additional maternal resources.

To be stable in evolutionary terms, signals must be an honest indicator of need. Otherwise, for example, if all offspring called at the same high rate whether or not they were in need, dams would come to ignore calling as deceptive. The theory of honest signaling implies a negative relationship between the signal and some aspect of signaler condition. Also to be beneficial, long-term signaling would have to secure some functional benefit for both the signaler and the primary receiver. Lastly, producing the signal itself must incur some cost. On this final point, vocalizing is presumed to be costly both in terms of physiological measures to produce the effort, but also in terms of putting the signaler at increased risk of predation, at least under natural conditions.

As with parent-offspring conflict, the theories of honest signaling have been evaluated in applied ethology studies of sows and piglets (Weary and Fraser, 1995). The evidence to date has shown the vocal response following weaning is more pronounced for young in greater need of maternal resources (e.g., milk) and this has been shown to be true for piglets (Weary and Fraser, 1995) and for calves (Thomas et al., 2001). Both of these studies were conducted on offspring that were very young and quite dependent on their dam for most of their nutrients through lactation, which is not necessarily the case for the offspring of all livestock species being weaned (e.g., beef calves).

Another prediction arising from the evaluation of behavioural responses as signals of need includes the notion that these responses should be increased if the signaler perceives that the intended receiver is nearby (see later discussion).

2.5 The consequences of artificial weaning and associated stressors

The extent of change in behaviour that follows artificial weaning provides evidence that this common practice causes animals to be in distress. And as previously stated, the scale of behavioural disruption caused by weaning may be unparalleled by any other management practice. Perhaps not surprisingly, the increased behavioural activity that characterizes an animal's response to weaning is associated with physiological changes that are also indicative of animals under stress (Lefcourt and Elsasser, 1995).

One important consequence of artificial weaning on animal production is that offspring tend to lose weight for some period of time after the event (Weary and Fraser, 1995). Further, the stress associated with artificial weaning has also been linked to immunosuppression (Griffin, 1989). Morbidity rates rise sharply among recently weaned calves (Harland et al., 1991) resulting in further losses through reduced performance and treatment costs. Mortality rates in calves caused by fibrinous pneumonia ("shipping fever") are higher in feedlots containing a large proportion of recently weaned calves (Harland et al., 1991). The effects of weaning stress on the subsequent health of calves may also be affected by other stressors commonly imposed at the same time including the co-mingling of calves from several sources (Ribble et al., 1995). Multiple stressors are known to have at least additive negative effects on the health and productivity of farmed animals (e.g., poultry: McFarlane et al., 1989; pigs: Hyun et al., 1998).

Artificial weaning also imposes social and environmental change upon dams and offspring. Often these changes are regarded as being more consequential for the young, but they may have important impacts on the dam as well. One important outcome is that the social relationship between dam and offspring is suddenly terminated. For the first time offspring may find themselves in a novel homogeneous social group, without any mature conspecifics present. Also, offspring are often mixed with unfamiliar animals, which causes some degree of social stress. From a nutritional point of view, milk is removed from the diet of the young and often replaced by dry feeds that may be unfamiliar. If the offspring are immediately marketed, as is commonly the case for newly weaned calves in North America, they may also spend a significant amount of time without any feed or water, plus the extra handling and transporting required contributes to additional stress (USDA-NAHMS, 1998). Further processing may also occur as part of preparations for marketing. For example, calves may undergo dehorning, castration, and vaccination. The dams likely experience some pain and discomfort related to the engorgement of their mammary glands with the sudden cessation of nursing.

Behavioural and physiological perturbations as well as reduced productivity and increased risk for health problems all offer evidence that current weaning practices compromise the welfare of farm animals. The various consequences and factors impacted by weaning have been studied in an attempt to understand how changing these might be used to reduce the stress associated with weaning.

2.6 The effects of age and nutrition on the response of offspring to weaning

2.6.1 Age effects on the response to weaning

One supposition underlying the current understanding of artificial weaning is that the distress response occurs at least partly because weaning is imposed prematurely relative to the natural weaning age. Gonyou and Stookey (1987) compared the artificial weaning age of various farmed animals to their ages of natural weaning. They calculated that the greatest deviation from the natural weaning age was seen in systems of swine production, based on an artificial weaning age of 21-35 d. Since that time the disparity has grown. Some swine systems practice segregated early weaning when piglets are just 10 d of age. Other livestock production systems have not followed this trend and therefore much of research that has investigated age effects on the response to weaning has been done in swine (e.g., Yuan et al., 2004; Mason et al., 2003). Fundamental research on the age effects for other species such as cattle would also answer important questions about whether, beyond the initial period of acute dependence, the response to weaning is lessened as the offspring approaches the natural weaning age. Investigating the influence of age at weaning may be of increasing importance with the increase in the number of studies investigating the early weaning of cattle (e.g., Myers et al., 1999). A major difficulty with studies examining age effects is they are often not discernable from the effects of nutrition in terms of milk intake. Also the younger offspring may be more dependent on the dam socially and psychologically, for example, for protection.

2.6.2 Nutritional effects on the response to weaning

Nutrition has been manipulated in studies related to weaning from a number of different perspectives. Nutrition of the dam has been manipulated to explore the possibility of accelerating the natural weaning process. One study which explored the factors involved in the natural weaning of sheep found that measures of the ewe-lamb bond diminished more rapidly for ewes fed on a low plane of nutrition to reduce their milk yield. The same ewes also weaned their lambs significantly earlier than ewes on a high plane of nutrition (Arnold et al., 1979).

Various nutritional management strategies have been tested in an attempt to reduce the negative response of the offspring to weaning. These have taken two primary approaches to ease the nutritional transition of the young following weaning, 1) reducing the milk availability prior to weaning (e.g., horses: Houpt et al., 1983; sheep: Arnold et al., 1979), and 2) increasing the consumption of concentrates or creep feed by the young prior to weaning (e.g., horses: McCall et al., 1985).

Lay et al. (1998) found that calves reared by restricted nursing (2 h/d) tended to differ in their response to weaning by abrupt separation compared with calves that nursed *ad libitum*, when both groups were weaned at 192 days of age. Calves raised on a restricted nursing schedule appeared to be less stressed by weaning as they vocalized less and walked less distance than controls. The restricted nursing rearing regime may have affected the attachment between cows and calves or simply reduced the degree of nutritional dependence. Houpt et al. (1983) tested the effects of varying the level of caloric intake by mares during lactation on the dam-offspring bond, measured by their response to separation. Mares fed the low-energy diet actually lost weight, but did not respond any differently to separation than mares fed higher diets of higher energy levels. The general activity of foals and their growth rate was similar across treatments when they were with their dams but they responded differently to separation. Decreasing the caloric content of the dam's diet resulted in foals walking less after weaning.

Affecting the dam's milk supply is one way to influence the amount of supplemental feed the offspring consume prior to weaning. Other more direct attempts have also been examined to increase supplemental intake by the young as a form of preconditioning to ease their transition after weaning. Foals preconditioned to creep feed have been found to spend more time standing and less time walking after separation compared to unconditioned foals, but their calling rate was not different from foals weaned without any prior conditioning in one study by McCall et al. (1985). McCall et al. (1987) then found that foals creep-fed prior to weaning gained more weight during the first 2 weeks after weaning, but that at 8 weeks after separation they were no different than foals weaned without creep feed prior to weaning. Hoffman et al. (1995) found foals raised at pasture with supplemental hay and concentrate vocalized less after weaning than foals that were raised at pasture with supplemental hay but without any concentrate suggesting the energy level of the preconditioning ration may be important.

Price et al. (2003) examined the effects of preconditioning on the behaviour of calves. The behaviour of calves preconditioned to alfalfa hay was similar to calves that were not preconditioned when both were weaned into dry lot pens. Although preconditioned calves did spend more time eating during the first three days after separation, their weight gain was no greater than controls, either at 2 or 10 weeks after weaning (Price et al., 2003). One issue preconditioning does not address is the fact that such strategies do little to alleviate the distress experienced by the dams.

Dairy calves weaned by the gradual dilution of their milk ration with water do not show the typical behaviour signs of weaning distress (Jasper et al., submitted). The authors have noted the more typical behavioural response by calves to weaning was elicited by removal of the feeding apparatus. This finding raises interesting questions about the possibility that the young may possibly form an attachment to the physical source of the milk since their behavioural response was negligible when the nutritional component of feeding was manipulated.

2.7 Abrupt weaning, by varying degrees of separation and fenceline contact

The most common method of forced weaning across species is by abruptly and permanently separating the dam and offspring. Often the separation is complete, preventing any visual and auditory contact. Livestock producers have remarked that complete separation of dams and offspring, by as much distance possible, hastens their recovery following weaning (Haigh et al., 1997). This may reduce the stress on fences that separate those animals highly motivated to reunite, but whether it reduces stress on the animals is questionable. However, we know that complete separation at weaning is an inherent aspect of beef production in North America. Surveys in the United States have found that at the point of separation for weaning more than half of all beef calves are shipped for marketing or feeding to a separate location (USDA-NAHMS, 1998).

A growing body of research shows that allowing dams and offspring fenceline contact after weaning, by separating them into adjacent pens, reduces their behavioural response. One of the earliest references on fenceline weaning showed calves gained more weight initially if they were fenceline weaned, but there was no long-term treatment benefit over weaning by remote separation (Nicol, 1977). The author additionally noted that calves in the fenceline group seemed less disturbed by the weaning process although behaviour was not formally recorded. The next reference reporting on this method showed that fenceline contact significantly affected the behaviour of foals immediately after separation, including reducing both their time spent walking and their rate of vocalizing compared to totally separated foals (McCall et al., 1985). Stookey et al. (1997) observed similar effects on the behaviour of calves and as with the study by Nicol (1977), a short-term benefit on weight gain.

The most recent and most thorough study published on fenceline weaning was done with cattle and it showed calves weaned by this method called half as much and spent half as much time walking as calves weaned by remote separation (Price et al., 2003). Fenceline calves also spent more time eating. It is interesting to note that fenceline calves behaved the same as unweaned controls in every respect except for their rate of vocalizing, which was significantly higher (Price et al., 2003). The study by Price et al. (2003) has been the only study to date to find long-term effects on productivity. Fenceline calves had a higher cumulative weight gain both 2 weeks and 10 weeks after weaning, compared to remotely separated calves (week 2: 21.4 vs. 11.0 kg; week 10: 50.0 vs. 38.2 kg).

Fenceline weaning has been shown to have similar behavioural effects on wapiti calves (Haigh et al., 1997). In this study dams and offspring in the control group were separated into paddocks just 50 m apart, prohibiting only visual contact. This raises the interesting question of what exactly constitutes "remote separation".

Research on red deer using modest numbers found that separating pairs by 100 m prolonged the behavioural response of calves compared to a separation distance of 2 km (Pollard and Littlejohn, 2000). The overall amount of time calves spent pacing and vocalizing was similar but tended to be less for calves separated by 2 km and their behaviour did return to baseline levels significantly sooner compared to calves separated from their dams by a distance of 100 m. Similarly, weaning ewes and lambs into paddocks 1.5 m apart, with relatively open fencing, Orgeur et al. (1999) found both the dams and their young vocalized significantly more than totally separated pairs on the third and fourth day following separation. These findings fit with hypotheses based on theories of honest signaling, which would predict vocalizing to be higher in situations where the young perceives the receiver, their dam, is aware of their vocal signal (Weary and Fraser, 1995). Furthermore, these results may be interpreted as fitting with the opinion of producers that the greatest possible distance of separation is recommended. Indeed if fenceline cannot be provided by separating dams and their offspring into directly adjacent pens, then it may well be better to impose weaning by total and remote separation.

Weaning with fenceline contact appears to have gained some popularity as an alternative and less stressful method than remote separation. This despite any physiological evidence to accompany the behavioural effects observed. Fenceline weaning does require that fences are well maintained and, compared to the common situation of weaning calves and sending them straight to a market, fenceline-weaned calves need to be specially managed for some period of time before they are shipped.

The benefits of weaning with fenceline contact on reducing the behavioural signs of distress leads to many interesting ethological questions that could be pursued to improve our understanding of the dam-offspring relationship and to better understand why the weaning process is so stressful. The fact that research suggests separating pairs into adjacent pens works best, raises questions about what qualities of fenceline contact are most important to achieve the beneficial effects. It is not clear whether actual physical contact is a critical element of this method although electric fencing, which presumably limits actual contact, has been used in these studies and proven effective (Price et al., 2003). Careful observations on the nature of interactions between dams and their offspring offered varying degrees of fenceline contact could prove to be quite revealing. Modifications of this situation could allow for isolation of various components of contact (visual, auditory, olfactory, tactile) to evaluate the importance of each one in reducing distress at weaning.

As changes in general activity and vocalizing are the primary responses to weaning only limited information is available about the dam-offspring behaviour, their interactions, their behavioural synchronicity, under conditions of fenceline contact. Price et al. (2003) reported that the proportion of time calves spent within 3 m of the fence on days 1-5 following separation was 62, 62, 46, 24, and 31%, respectively, while the dams were 38, 45, 26, 12, and 3%, respectively. The authors noted that cows udders were most distended on d 2. These data may offer insight into the dissolution of the dam-offspring relationship in terms of how the behavioural responses might relate to physiological changes.

2.8 Effects of the social environment on the response to weaning

As mentioned previously the offspring of most farmed animals experience dramatic changes in their social environment following weaning. Firstly they lose social contact with their dam, and second, for the very first time in their lives, they may be kept in an adult-free homogeneous group and mixed with unfamiliar conspecifics.

Generally few farm animal species are ever weaned under conditions of complete social isolation. However, sometimes foals are weaned this way for fear they may become overly aggressive or suffer injuries by their interactions (Heleski et al., 2002). Houpt et al. (1984) found that immediately after separation, 12 h after separation, and 1 week later, foals weaned individually vocalized at a rate about twice that of foals weaned into similar conditions but housed in pairs. It may have been that some of the vocalizing was due to maternal separation and some vocalizing may have been a residual response to social isolation more generally, since they could hear and presumably smell, but not see, other foals. Malinowski et al (1990) found that although weaning elevated the plasma cortisol levels of foals compared to their baseline measures no treatment difference was found between single- or pair-housed foals. On the other hand Hoffman et al. (1995) used a combined behaviour scoring system and concluded that foals weaned singly were less stressed than foals weaned in pairs. Hoffman et al. (1995) observed a trend for lower vocalization rates in paired foals as did Houpt (1984), but their explanation was that these foals engaged in aggressive interactions thus reducing the time available for vocalizing. Single-housed foals spent more time standing. It is unclear whether foals in this study were familiar with one another. It seems unlikely that a social animal would better cope with weaning by being kept totally isolated. However, from the standpoint of reducing aggressive bahaviour and reducing the risk of animals injuring each other, this option may be sound advice.

More fundamental work on the effects of the social environment on separation from the dam has been done with sheep. Porter et al. (1995) found that lambs separated from their dam called more if they were isolated than if they were paired with another lamb. Furthermore, lambs called less when paired with their twin than with an unfamiliar lamb. This work has not been repeated to assess the effects of social environment at the time of weaning although it raises very interesting questions.

One way to minimize the changes experienced by offspring at weaning is to only remove their dam from the social environment. Church and Hudson (1999) explored this method with wapiti calves by examining the effects of removing a few dams from the social group each day over a 2-week period. Unfortunately, the discrete effects of this method were confounded by the fact that dams removed a few at a time were then placed into adjacent pens thus offering fenceline contact where the controls were remotely separated. To date this technique has not been repeated in any scientific trial although it raises the interesting question about whether the level of distress of the social group itself might affect the response of the weaned individuals.

The response of calves to weaning in isolation has not been compared with weaning them in pairs because such weaning protocols have very little practical application for cattle production. However, answers to such questions, including whether the behavioural response of calves to weaning differs when they are in entirely familiar or co-mingled groups could help resolve important questions about the importance of the social environment effects. It has been well documented that weaning causes disruption to social organization of calves (Veissier and le Neindre, 1989). Newly weaned calves group together more and also engage in more social encounters than calves that are still able to nurse. Interestingly, this has been interpreted as a strengthening of the social bonds among newly weaned calves (Veissier and le Neindre, 1989) although no experiments have been done to directly test these supposedly strengthened social bonds.

2.8.1 The use of companion or trainer animals

In North America it is common that calves are separated from their dams for weaning and then immediately marketed and assembled into large, co-mingled groups (e.g., 250 animals) in dry-lot environments. Regarding the effects of a novel environment, Price et al. (2003) reported that calves in dry-lots walked less than calves at pasture. A more vital issue from the perspective of animal production is that feedlot managers have regarded the low feed intake and lack of feeding behaviour as an indication that calves are unable to readily locate the feeders and waterers in their new environment.

Research has been conducted to investigate whether calves would adapt better to a new feedlot environment sooner if there were some resident cattle already in those dry lot pens (Gibb et al., 2000; Loerch and Fluharty, 2000). Gibb et al. (2000) found the presence of a pregnant "trainer" cow in each pen of 126 newly weaned calves had no beneficial effects on the health of calves, the time they spent eating or their weight gain. Loerch and Fluharty (2000) used trainer cows and steers and found this benefited weight gained by calves in three of four trials at the level $P<0.06$. Time spent eating was similar for pens whether or not trainer companions were present and in fact in the only trial where pens with resident companions did spend more time eating, there was no overall difference in weight gain (Loerch and Fluharty, 2000).

These results suggest having occupant animals already present in dry-lot pens when newly weaned calves arrive does not obviously facilitate their transition in terms of reducing calling or walking or in terms of increasing time spent eating or weight gain (consistently). However, one issue not addressed by these studies was that the companion animals used were unfamiliar to the calves and so they may have, in fact, been avoided by the newly arrived calves.

A modified version of this concept has been investigated by housing groups of 12 farmed red deer calves with two hand-reared adult hinds, after they were separated from their dams for weaning (Pollard et al., 1992). No differences in the general activity of calves could be attributed to the weaning treatment. However, during the first 7 d after separation calves housed together with hinds gained more weight than controls although, curiously, there was no treatment effect over the first 14 d in that trial (Pollard et al., 1992).

2.8.2 Minimizing disruption to the social environment: Criss-cross weaning

One weaning method that truly minimizes the degree of social change that calves experience at weaning has been called criss-cross weaning (Nicol, 1977). By this method a cowherd is split into two groups and their calves exchanged and thus calves are weaned in the presence of both familiar cows and calves with the major change being the absence of the nursing partner. The one and only study to investigate criss-cross weaning to date evaluated the weight gain of calves alone, and found no benefit of this method compared to abrupt and distant separation (Nicol, 1977). No results or even impressions about the behavioural response of cattle to this method were noted.

2.9 Progressive or gradual weaning

One belief underlying the perception about natural weaning is that the process is very gradual and this has led to some attempts to impose artificial weaning gradually as well. Emphasizing the alleged importance of breaking the dam-offspring bond methods of progressive or gradual weaning have tried to employ repeated and often successively longer periods of dam-offspring separation prior to the point of permanent separation. This method of preparation for weaning could theoretically serve to reduce the dam-offspring bond or to reduce the dam's milk yield (by reducing nursing frequency), or both.

The dams and offspring of various species (e.g., horses: Moons et al., 2005; sheep: Orgeur et al., 1998) have been repeatedly separated for successively longer and longer periods leading up to the final, permanent, separation. To date this technique has not been tried with cattle. Moons et al. (2005) found that separating mares and foals for 10-min at 2, 4, 6, 8, 10 and 12 weeks of age had no effect on their response to weaning at 24 weeks of age, compared to controls.

In a study not designed to investigate weaning per se, Cockram et al. (1993) evaluated the response of ewes repeatedly separated for 3 h and then reunited with their lambs twice daily for 23 d beginning when lambs were 2 weeks of age. The authors found ewes repeatedly separated responded similarly to those repeated separations. Those ewes also responded the same to a permanent separation test imposed after the 23-d period. Their results suggest there was no habituation to this process of separation, by the ewes, at least up to 23 d after giving birth. In a study employing separations over a long period of time, Orgeur et al. (1998) looked at the daily separation of lambs and ewes starting at 3.5 weeks of age and increasing the duration of time away until their final permanent separation for weaning at 12 weeks of age. Based on the behavioural response the authors reported habituation to the repeated separations. They found the number of high-pitched bleats by progressively weaned lambs was lower than for abruptly weaned lambs following weaning. Based on the results presented in their paper it would appear that during this habituation process, the cumulative behavioural response of ewes and lambs to the multiple and repeated separations, likely far exceeded the behavioural response of control animals to weaning. Unfortunately, the data was not presented in this way.

Studies of imposing repeatedly separating dams and offspring often do not explicitly state the rationale as to what this kind of treatment is designed to achieve or why these techniques might be effective. It could be hypothesized that the goal of repeated separations is to diminish the dam-offspring bond or to habituate their respective responses to separation. At the same time, depending on their frequency and duration, separations may serve to reduce the nursing frequency and so reduce the milk production of the dam while promoting the young to increase their nutritional independence during their time away from the dam.

2.10 Future research needs

Investigations into the stress and behavioural responses associated with weaning have been the domain of applied scientists. As such the literature points to a focus on developing methods that reduce the behavioural response and presumably the underlying stress. However, the essential preceding basic research with farm animal species has not received adequate attention, perhaps with swine as an exception.

3.0 WEANING CATTLE IN TWO STAGES CAUSES FEWER BEHAVIOURAL SIGNS OF DISTRESS THAN WEANING ABRUPTLY, BY SEPARATION

3.1 ABSTRACT

Weaning imposed by the separation of dam and offspring terminates milk feeding but also prevents other forms of physical and social interaction. In this study, I isolated the effects of terminating nursing from the effects of physically separating cows and calves to determine how these two factors affect the behavioural response of beef cattle when weaning is imposed. Eight of 16 cow-calf pairs were weaned by an experimental two-stage procedure, which enabled me to first measure their response to the termination of nursing (Stage 1) and then measure their subsequent response to being physically separated (Stage 2). For Stage 1, calves wore an antisucking device that prevented nursing but allowed them to be together with their dam. Control pairs were able to nurse up to separation, the same point in time that two-stage pairs were separated. Behaviour of all pairs was recorded for 12 h/d throughout the study: a baseline period (2 d), Stage 1 (5 d) and Stage 2 (4 d, following separation). When two-stage pairs were prevented from nursing only very slight changes in behaviour were observed, but their subsequent response to separation was greatly reduced compared to abruptly weaned controls. Compared to control dams, over the 4 d after separation, two-stage cows vocalized 70% less frequently (19.2 vs. 62.2 calls/h; $P<0.01$) and on average spent 79.2 additional min/d eating (280.0 vs. 200.8 min/d; $P<0.05$). Calves weaned in two stages vocalized

85% less than control calves after separation (7.2 vs. 48.3 calls/h; $P<0.01$). Time spent walking by control calves after separation was 83.5 min/d, while calves weaned in two stages walked for 16.9 min/d, about 80% less ($P<0.0001$). These milder behavioural responses by pairs weaned in two stages suggests they were less distressed than controls weaned by separation. Allowing for continued physical interaction between cows and calves as nursing is terminated, as occurs in natural weaning may account for the reduced behavioural response by cattle weaned in two stages. A two-stage approach may offer a practical technique for weaning cattle and similar principles to reduce the stress of forced weaning might apply to other livestock species.

3.2 INTRODUCTION

Information about natural weaning in many farmed animal species is quite limited (cattle: Reinhardt and Reinhardt, 1981; pigs: Jensen, 1986; sheep: Arnold et al., 1979). This is due in part to the fact that under many livestock production systems weaning is imposed artificially prior to completion of the natural process. Additionally, because natural weaning occurs gradually, an accurate description of the process requires considerable research effort.

Weaning may result in increased autonomy of the young (e.g., independent foraging, independence from protection by the dam) but it is commonly defined as the cessation of suckling for milk (see Martin, 1984). Descriptions of developmental changes in suckling behaviour (e.g., frequency and duration of nursing) are available for many species. However, this information offers little insight into the timing or progression of natural weaning because these basic measures correlate poorly with milk transfer (Cameron, 1998). Measured directly, milk yield has been established as an important determinant in the natural weaning process in sheep (Arnold et al., 1979). Ewes that produce less milk wean their young earlier. Well-fed ewes will wean later than poorly fed ewes.

Reinhardt and Reinhardt (1981) reported that natural weaning (cessation of nursing) in Zebu cattle (*Bos indicus*) occurred when calves were between 7 and 14 months of age. The authors reported that weaning actually occurred over a two-week period. They also observed that calves were prevented from nursing by their dams, and that female calves were weaned earlier than males (on average 8.8 vs. 11.2 months of age). It has been hypothesized that natural weaning is regulated by parent-offspring conflict (Trivers, 1974), and observations that cows actively resist persistent suckling attempts by their calves (Reinhardt and Reinhardt, 1981) provide some support for Trivers' theory.

Beef cattle in North America are typically weaned when calves are between 6 and 9 months of age. The most common method involves the abrupt, permanent and often distant separation of cows and calves. Forced weaning disrupts the normal behaviour of both the cow and calf in highly predictable ways. After separation cows and calves show increased rates of vocalizing and they also spend more time walking (Veissier and Le Neindre, 1989; Veissier et al., 1989a). They also spend less time eating and resting after separation (Veissier and Le Neindre, 1989a; Veissier et al., 1989). These behaviour changes relative to baseline activity have been reported to last for three days following the separation of dam and young (Veissier et al., 1989; Stookey et al., 1997).

The remarkable deviations from normal behaviour, and in particular the increased rate of vocalizing, represent the animal's overt biological commentary on the negative effects they experience as a result of being weaned by separation. Physiological measures such as increased concentrations of circulating catecholamines indicate physiological stress (Lefcourt and Elsasser, 1995). The stress of weaning is recognized as one factor which might contribute to a stress-induced immunosuppression and increased risk for the development of respiratory diseases in feedlot calves (Wieringa et al., 1974; Harland et al., 1991).

Weaning by separation has the desired effect of terminating milk feeding, but it also disrupts other forms of physical interaction and communication between cows and their calves. Bison dams and their offspring maintain close social associations even after natural weaning (Green et al. 1989). These close associations have also been shown to persist in cattle, even after the dam gives birth to subsequent offspring (Reinhardt and Reinhardt, 1981). One alternative to the distant separation of cows and calves, weaning by fenceline contact, allows cows and calves to maintain a closer proximity after weaning and has been shown to reduce changes in their behaviour (Stookey et al., 1997; Price et al., 2003).

The reaction of beef cattle to abrupt weaning, imposed by separation, might be more predominantly affected by the termination of nursing (a nutritional factor for the calf, a physical factor for the dam) or possibly by the separation of cows and calves (a social factor for both cows and calves). The effects of these two factors are often confounded in studies investigating the effects of weaning. The purpose of this study was to measure the responses of cows and calves to weaning by sequentially isolating the effects of terminating nursing and physically separating cows and calves.

3.3 MATERIALS AND METHODS

All experimental procedures used in this study were approved by the University of Saskatchewan's Committee on Animal Care and Supply (UCACS Protocol #20000096; CCAC) and animals were cared for according to Guidelines set by the Canadian Council on Animal Care (1993).

3.3.1 Animals

Sixteen crossbred cows and their calves were used. Fifteen cows had single calves and one had twins. Cows were primarily of Charolais, Hereford and Simmental breed origins. Their calves were sired either by Charolais or Hereford bulls. At the beginning of this study, cows were (mean ± S.D.) 2.3 ± 1.8 years of age (range=2 to 9 years of age) and on average, in their second parity (range=1 to 8). Calves were 224 ± 5 d of age on the day they were separated from their dams.

Pairs were moved from pasture to dry-lot pens (30.5 × 27.5 m) where they were fed a diet of free-choice hay with water available *ad libitum*. They adapted to these conditions for 10 days prior to the start of observations.

3.3.2 Treatments and experimental design

Pairs were randomly assigned to one of two treatments. Half the pairs (n=8) were weaned by an experimental two-stage procedure designed to isolate the effects of terminating nursing from the effects caused by separating cows and calves. During Stage 1, calves wore an antisucking device (Figure 3.1), to prevent nursing for 5 d. The device hung from the calf's nose and prevented nursing by covering the calf's mouth as the head was positioned to nurse. Calves were able to eat, and drink water while wearing the device. Cows and calves were able to have physical and social interactions apart from nursing. Stage 2 of the experimental procedure involved the physical separation of cows and calves. The remaining pairs were controls (n=8) and were weaned by the method commonly used in beef cattle production systems, separating the cows and calves (terminating nursing and social contact at the same time).

The separation of cows and calves from both treatment groups occurred at the same point in time. Thus control pairs did nurse for 4 more days than pairs being weaned in two stages. Details of the experimental timeline are provided in Figure 3.2.

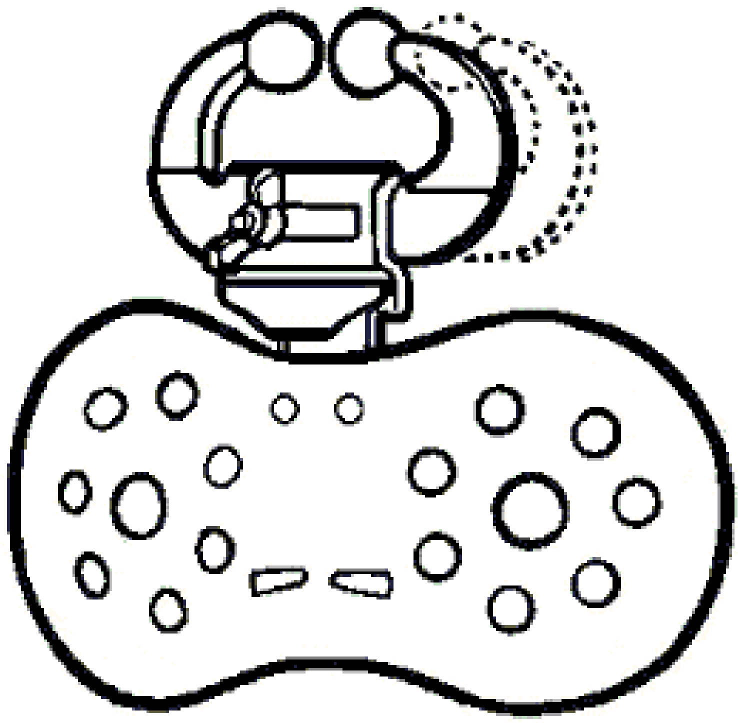


Figure 3.1. Diagram showing the basic design of the antisucking device worn by two-stage calves to prevent nursing. The device was manufactured from galvanized steel, and the nose inserts were tipped with rounded polypropylene ends. Devices acted as a physical barrier, which stopped calves from getting a teat into their mouth, but the device still allowed eating, drinking and additionally allowed calves to interaction with their dam. Use of the device did not result in cows actively rejecting nursing attempts. The dotted lines show how the gap of the nosepiece expanded by sliding open for application of the device. After it was fitted, the nose-gap was set by hand-tightening a butterfly-nut.

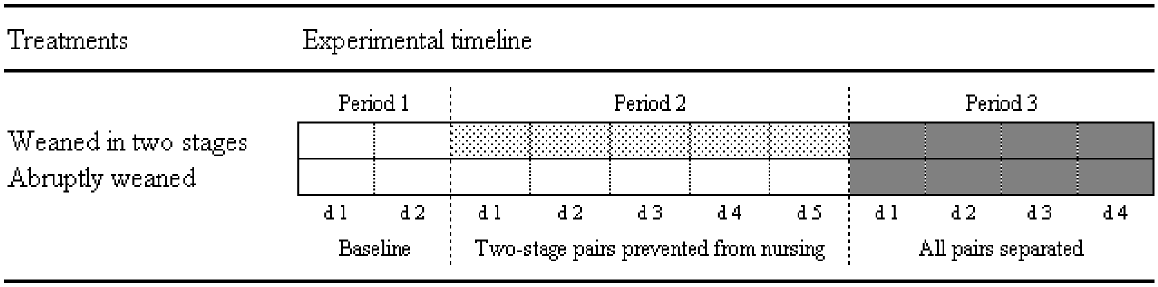


Figure 3.2. The treatments and the experimental timeline for the present experiment, illustrating the days when cows and calves were nursing, days when two-stage cows and calves were together, but prevented from nursing, and days when the cows and calves from both treatment groups were apart, and observed.

This study was carried out in two successive trials, each with 8 cow-calf pairs (4/treatment). Treatments were housed together in the same pen. At the point of separation both the cows and the calves were moved to new pens. The dimensions and basic layout of the new pens were similar to their previous housing conditions, but the new enclosures were approximately 60 m apart, within auditory range but precluding visual or physical contact between the cows and calves.

3.3.3 Behaviour observations

Behaviour was recorded during daylight hours from 0700-1900 h, 12 h/d for 11 d (see Figure 3.2). Cows and calves were numbered with livestock paint for easy identification from a distance. The behaviour of each individual animal was recorded. Before separation, a single observer was able to record the behaviour of all the cows and calves. Separation resulted in the formation of two pens and two observers were needed to record behaviour. Observers balanced the time they spent watching each of the two pens.

Instantaneous scan sampling was used to record general activity every 5 min. At each interval, an observer recorded the appropriate behaviour states of every animal: lying, walking, drinking, eating, nursing, ruminating, and grooming. These behaviour categories were not all mutually exclusive, for example a cow could be ruminating while simultaneously nursing her calf. During the period that two-stage calves wore the antisucking device the time spent attempting to nurse was recorded by the same instantaneous scan sampling method. A “nursing attempt” was defined as the calf having its head or muzzle in contact with dam’s udder.

At each 5-min interval, after recording the behaviour states, the number of vocalizations made by every individual during a continuous 2-min period were recorded. Any audible vocal sound that could be attributed to a specific individual was counted as a vocalization. Bursts of vocalizing were recorded by counting the number of individual short successive calls within each sequence. These were distinguished by inhalations taken by the animal between each call (see Kiley, 1972, See-saw calls - type B, p. 193).

Aggressive acts and were recorded continuously and the identification of the aggressor and the receiver was noted. To reduce ambiguity about aggressive behaviour by threats, aggression was operationally defined as a head-butt that made physical contact with the target animal and which caused the recipient to move from its initial position.

The same observation methods were used on every day of the experiment. Prior to separation a single observer was able to record the behaviour of all animals. After pairs were separated it was necessary to have two observers (one per pen). Observers switched at regular intervals to balance their recording between the two pens.

3.3.4 Statistical analysis

The rate of vocalizing from sampling intervals was used to estimate the total number of calls/h for each individual animal, on each day of observation. The total time (min/12 h observed) that animals spent in behaviour states on any given day was calculated based on the percentage of sampling intervals each state was observed, multiplied by the total number of minutes observed on that particular day. Continuously recorded acts of aggression were expressed in terms of their frequency rate (acts/h).

All data were analyzed using a generalized estimating equations (GEE) method to account for repeated measures within cow or calf. Data were analyzed using a statistical computer software program; SAS v.8.2 for Windows (SAS Institute, Cary, North Carolina, USA, 1997). Analysis used the GENMOD procedure of SAS. Model specifications included a normal distribution, identity link function, repeated statement with subject equal to calf identification, and an AR(1) correlation structure. Variables remaining in the final multivariable model at $P<0.05$, based on the robust empirical standard errors produced by the GEE analysis, were considered statistically significant.

Treatment effects and day effects were analyzed within 3 specific time periods: 1) the baseline period when all pairs were nursing, 2) Stage 1, the period when two-stage pairs were prevented from nursing, and 3) Stage 2, the period after cows and calves were separated. The fact was considered that two-stage pairs were being weaned over a longer period of time than controls (e.g., 5 d without nursing + 4 d of separation vs. 4 d of separation, respectively). To help gauge whether the response of two-stage animals to weaning was merely the same response diluted over time the overall response of cows and calves from both treatment groups their behaviour was also compared 4) across time periods 2 and 3.

The association between both day and treatment, and each behaviour, were first examined alone. If both of these factors were significant then treatment and day were examined together with the treatment × day interaction term. If the interaction term was significant then treatment effects were examined on individual days. In all cases abrupt weaning acted as the reference group.

The question of whether animals weaned in two stages responded more to the termination of nursing or to physical separation from their partner, was explored by comparing their respective behaviour during stages 1 and 2. The same number of days were analyzed from each time period: the response of animals on the first 4 d nursing was prevented was compared to their response on the 4 d observed following separation.

Stage 1 of the second replicate was abbreviated to 3 d because the particular antisucking devices were not staying in place reliably. To avoid handling calves repeatedly to replace the devices, the period that nursing was deprived was reduced to 3 d. Henceforth the data are referred to as Trial 1 and Trial 2.

Data from Trial 2 were analyzed separately using the same statistical methods described previously. Results are presented with separate references to the two trials. The twin calves mentioned previously were assigned to different treatments, thus in Trial 2, 5 calves were weaned in two stages and 4 calves were weaned by separation.

3.4 RESULTS

Complete behaviour results (mean ± SE) and all levels of significance for Trial 1 and Trial 2 are presented in Table 3.1 (A: cows, B: calves).

3.4.1 Period 1 (baseline)

The baseline period was primarily used to confirm that all pairs to be weaned were indeed nursing and this was verified. In Trial 1 there was a treatment difference between the time cows spent eating (P<0.05) and ruminating (P<0.001). In Trial 2 cows assigned to the two-stage treatment spent less time nursing their calves (13.1 vs. 19.4 min/d; P<0.05). The only behaviour difference between calf groups was seen in Trial 2 where calves to be weaned in two stages spent more time lying (P<0.01; see Table 3.1-B).

3.4.2 Period 2 (Stage 1: Trial 1=5 d, Trial 2=3 d)

Preventing nursing had few effects on the behaviour of cows and calves, based on the variables recorded and there were some differences in response between Trials 1 and 2.

Table 3.1. Mean (± SE) behaviour values for cows (A) and calves (B) weaned in two stages or by separation alone. The rate of vocalizing is expressed in calls/h, aggression as the number of acts/h and other activities as min/d (min per 12-h observed). Analyses were performed within specific time periods: 1) baseline period when all pairs were nursing, 2) the period when two-stage pairs were prevented from nursing, 3) the period after cows and calves were separated. To gauge the overall response of cows and calves to weaning, an overall treatment comparison was performed combining periods 2) and 3) above. Treatment means separated by * differ by $P<0.05$; ** by $P<0.01$; *** by $P<0.001$; or by the actual P=value listed to indicate a statistical trend. Trial 1 and Trial 2 were analyzed separately.

A	COWS					
	Trial 1			Trial 2		
Period Behaviour	Two stage weaning 5 d	*P*-value	Abrupt weaning	Two stage weaning 3 d	*P*-value	Abrupt weaning
Period 1 (baseline period)						
Vocalizing	0.0 ± 0.0		0.3 ± 0.1	0.3 ± 0.3		0.8 ± 0.3
Walking	6.3 ± 3.2		8.8 ± 1.3	12.5 ± 3.4		7.5 ± 0.9
Eating	364.4 ± 21.0	**	317.5 ± 20.9	271.9 ± 20.0		248.7 ± 19.3
Lying	163.8 ± 23.5		160.0 ± 24.3	84.4 ± 15.3		106.9 ± 15.8
Nursing	10.6 ± 1.1		12.5 ± 3.1	13.1 ± 2.3	*	19.4 ± 2.0
Grooming	8.1 ± 3.3		6.9 ± 3.8	2.5 ± 0.9		1.9 ± 0.9
Aggression	0.5 ± 0.1		0.6 ± 0.2	0.4 ± 0.2		0.3 ± 0.1
Ruminating	58.8 ± 11.4	***	104.4 ± 19.6	111.9 ± 13.1		120.0 ± 13.7
Period 2 (two-stage pairs prevented from nursing)						
Vocalizing	4.2 ± 2.2	*	0.6 ± 0.2	11.9 ± 8.6	0.09	0.4 ± 0.1
Walking	8.2 ± 1.9		7.5 ± 1.4	15.8 ± 2.7	*	7.5 ± 2.0
Eating	270.0 ± 20.5		239.8 ± 20.7	213.7 ± 17.4		209.6 ± 13.8
Lying	123.7 ± 14.8		129.7 ± 17.5	103.3 ± 11.6		113.8 ± 16.4
Nursing	2.8 ± 0.9	***	10.5 ± 1.6	2.1 ± 1.1	**	14.2 ± 3.6
Grooming	8.3 ± 1.7	*	5.5 ± 1.3	6.7 ± 2.2		6.7 ± 1.5
Aggression	0.4 ± 0.1		0.5 ± 0.1	0.2 ± 0.0		0.2 ± 0.1
Ruminating	70.7 ± 7.9	0.06	92.5 ± 11.6	110.0 ± 11.1	0.08	127.5 ± 8.8
Period 3 (all pairs separated)						
Vocalizing	16.6 ± 8.4	***	66.7 ± 13.0	21.8 ± 7.6	**	57.6 ± 16.5
Walking	16.0 ± 3.0	0.06	26.9 ± 4.5	15.9 ± 2.6	***	29.4 ± 5.4
Eating	278.5 ± 15.5	***	203.1 ± 11.0	281.3 ± 13.0	***	198.4 ± 17.4
Lying	114.9 ± 15.4		134.1 ± 18.8	52.8 ± 11.3	***	89.4 ± 16.8
Grooming	0.9 ± 0.5		1.2 ± 0.7	1.3 ± 0.6		0.9 ± 0.7
Aggression	0.2 ± 0.1	*	0.4 ± 0.1	0.2 ± 0.1		0.4 ± 0.1
Ruminating	53.2 ± 8.6		69.1 ± 9.1	56.9 ± 8.8		57.8 ± 9.9

B	CALVES					
	Trial 1			Trial 2		
Period Behaviour	Two stage weaning 5 d	*P*-value	Abrupt weaning	Two stage weaning 3 d	*P*-value	Abrupt weaning
Period 1 (baseline period)						
Vocalizing	0.0 ± 0.0		0.0 ± 0.0	0.0 ± 0.0		0.1 ± 0.0
Walking	5.6 ± 1.1		10.0 ± 3.4	17.5 ± 3.1		13.1 ± 3.3
Eating	302.5 ± 19.8		281.9 ± 22.6	256.5 ± 20.3	0.06	296.9 ± 9.3
Lying	241.2 ± 20.9		246.2 ± 23.5	159.5 ± 12.5	**	123.1 ± 9.5
Nursing	21.9 ± 3.0		23.1 ± 2.5	13.5 ± 1.7		15.0 ± 1.9
Grooming	3.1 ± 1.6		1.3 ± 0.8	0.5 ± 0.5		0.6 ± 0.6
Aggression	0.1 ± 0.0		0.1 ± 0.1	0.0 ± 0.0		0.0 ± 0.0
Ruminating	93.7 ± 19.3		76.2 ± 13.4	92.5 ± 10.5		73.8 ± 8.5
Period 2 (two-stage pairs prevented from nursing)						
Vocalizing	0.5 ± 0.2		0.8 ± 0.5	1.1 ± 0.5	*	0.2 ± 0.1
Walking	9.5 ± 1.9	**	4.8 ± 1.1	12.3 ± 2.8	0.16	8.7 ± 1.6
Eating	231.3 ± 16.2	0.17	244.2 ± 19.5	155.7 ± 13.2		167.9 ± 16.1
Lying	190.5 ± 19.5		179.3 ± 16.4	160.7 ± 9.2	**	178.7 ± 15.6
Nursing	10.8 ± 2.3		10.0 ± 2.3	9.0 ± 2.2		12.9 ± 4.1
Grooming	1.0 ± 0.6		1.3 ± 0.7	0.3 ± 0.3	**	2.1 ± 1.1
Aggression	0.03 ± 0.0	0.06	0.07 ± 0.0	0.00 ± 0.0	*	0.02 ± 0.0
Ruminating	74.8 ± 8.3		76.7 ± 8.0	90.0 ± 10.7	0.18	103.7 ± 11.7
Period 3 (all pairs separated)						
Vocalizing	3.4 ± 1.0	***	44.2 ± 9.8	10.9 ± 4.3	**	52.4 ± 13.2
Walking	15.3 ± 2.4	***	99.4 ± 19.2	18.5 ± 2.7	***	67.5 ± 14.4
Eating	213.1 ± 17.2	***	148.1 ± 14.8	238.3 ± 10.6	**	189.1 ± 15.6
Lying	215.3 ± 28.3	**	175.6 ± 26.0	113.5 ± 10.6		95.6 ± 16.1
Grooming	0.0 ± 0.0		0.0 ± 0.0	0.0 ± 0.0		0.3 ± 0.3
Aggression	0.3 ± 0.1	*	0.9 ± 0.3	0.2 ± 0.1	*	0.5 ± 0.1
Ruminating	60.9 ± 10.0	0.10	36.6 ± 10.7	62.3 ± 6.1	0.15	45.6 ± 7.4

In Trial 1, cows prevented from nursing had a higher rate of calling than controls (4.2 vs. 0.6 call/h; $P<0.05$) and there was a statistical trend for the same result in Trial 2 ($P<0.09$). Cows prevented from nursing spent more time grooming their calves than controls, but only in Trial 1 ($P<0.05$).

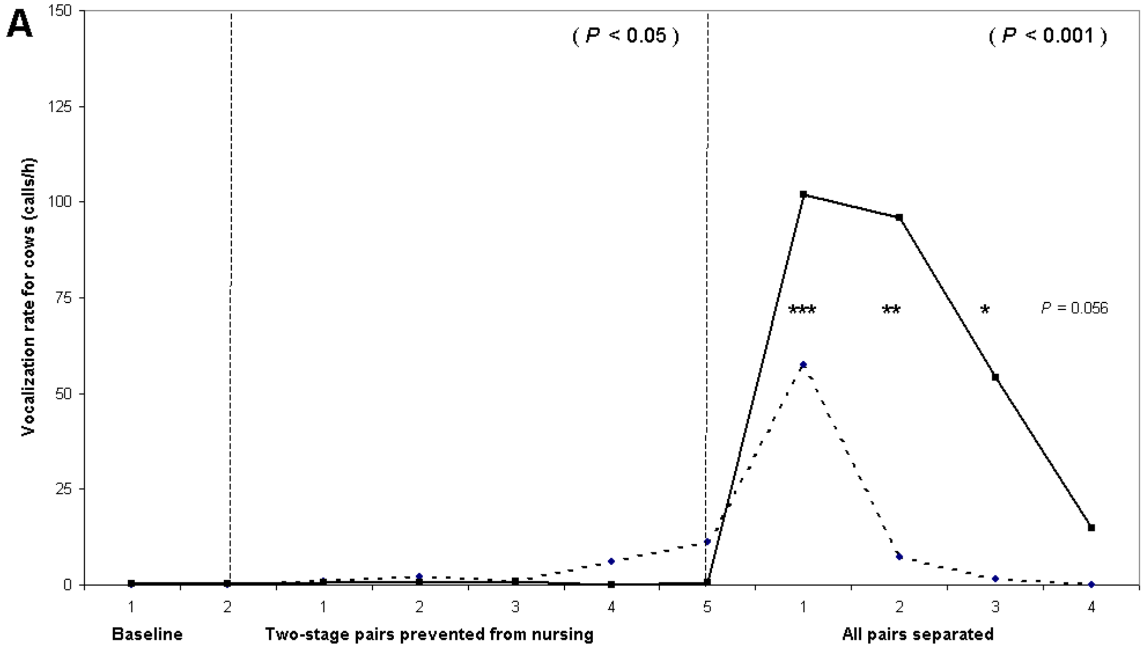
Calves prevented from nursing their dams did not differ in the amount of time spent eating hay, compared to control calves that were still able to nurse ($P>0.10$). Preventing nursing caused two-stage calves to be more vocal than controls, but only in Trial 2 and the rate of vocalizing was relatively low (1.1 vs. 0.2 calls/h; $P<0.05$). Preventing nursing also increased the amount of time two-stage calves spent walking compared to controls, but only in Trial 1 (9.5 vs. 5.8 min/d; $P<0.01$).

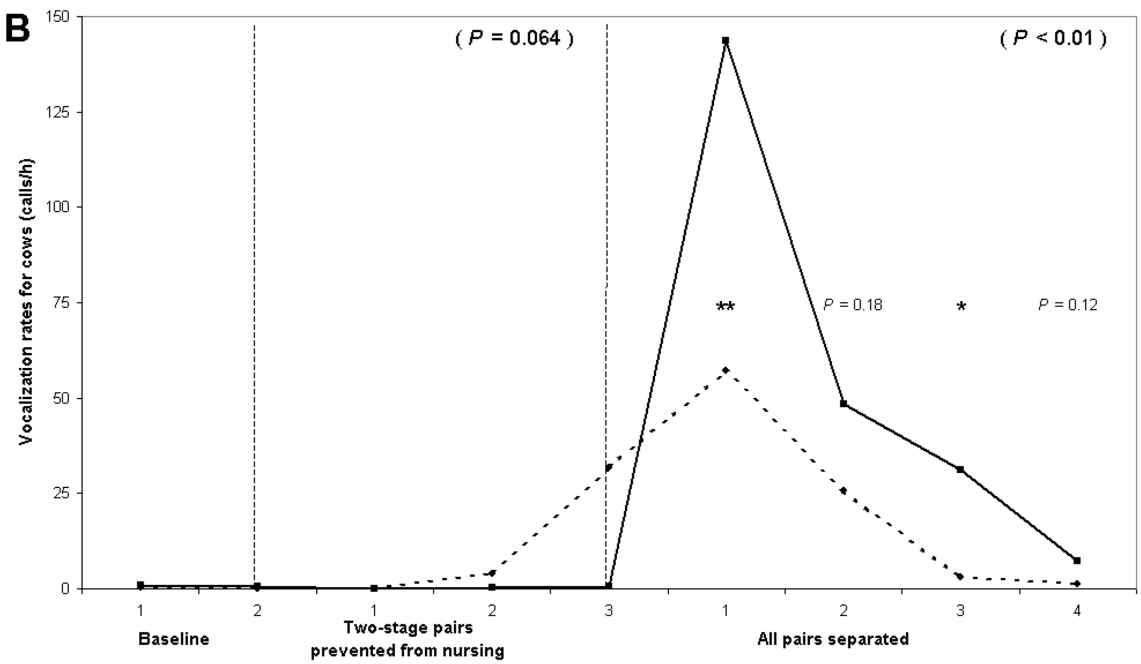
3.4.3 Period 3 (Stage 2: following separation)

Two-stage cows vocalized less than controls in response to separation; producing, on average, 70% fewer calls than controls ($P<0.001$). Calling by cows was highest on the first day of separation and it declined thereafter although treatment differences persisted even on the fourth day following separation in Trial 1 ($P<0.05$), and tended to be different on the fourth day in Trial 2 ($P=0.131$). Mean rates of vocalizing are illustrated in Figure 3.3 (A=Trial 1; B=Trial 2).

Two-stage cows tended to walk less than control cows in Trial 1 (16.0 vs. 26.9 min/d; $P=0.063$) and there was a significant treatment effect on walking by cows in Trial 2 with

Figure 3.3. Mean (±SD) vocalization rates[1] for cows (calls/h) on each day of the experiment. Cows were either weaned in two-stages - - -●- - - (nursing deprived for 5 d [A] or 3 d [B] prior to separation), or abruptly weaned —■— . Italicized *P*-values in parentheses indicate significant treatment effects within each period. Treatment effects on specific days of the experiment are shown by asterisks: *P*<0.05 (*), *P*<0.01 (**) and *P*<0.001 (***). Statistical tendencies on specific days are indicated by the actual *P*-value. [1]Call rates were calculated based on the number of calls recorded during a 2-min sampling period, taken every 5 min during daylight hours.


A
150
125
100
75
50
25
0
Vocalization rate for cows (calls/h)
(P < 0.05)
(P < 0.001)
***
**
*
P = 0.056
1
2
1
2
3
4
5
1
2
3
4
Baseline
Two-stage pairs prevented from nursing
All pairs separated


B
150
125
100
75
50
25
0
Vocalization rates for cows (calls/h)
(P = 0.064)
(P < 0.01)
**
P = 0.18
*
P = 0.12
1
2
1
2
3
1
2
3
4
Baseline
Two-stage pairs
prevented from nursing
All pairs separated

two-stage cows spending half as much time walking as abruptly weaned cows (15.9 vs. 29.5 min/d; *P*<0.001). In both trials, two-stage cows spent more time eating over the four days following separation, compared to controls (Trial 1: 37% higher, 75.4 additional min/d, *P*<0.001; Trial 2: 42% higher, 82.9 additional min/d over controls, *P*<0.001).

Abruptly weaned cows were more aggressive than two-stage cows following separation, but only in Trial 1 (*P*<0.05).

In both trials, after separation, the rate of vocalizing was lower for two-stage calves (Trial 1: 92% lower than controls, 3.4 vs. 44.2 calls/h, *P*<0.001; Trial 2: 79% lower than controls, 10.9 vs. 52.4 calls/h; *P*<0.001). The daily rate of vocalizing for calves across the entire study period is shown in Figure 3.4 (panels A and B show the results from Trials 1 and 2 respectively). The treatment effect on vocalizing by calves was still significant four days after separation in Trial 1 (*P*<0.0001). In Trial 1, calling by calves peaked on the second day of separation, whereas calling by calves peaked on the first day of separation in Trial 2.

Two-stage calves spent less time walking after separation than controls (Trial 1: 85% less time, 15.3 vs. 99.4 min/d, *P*<0.001; Trial 2: 73% less time, 18.5 vs. 67.5 min/d, *P*<0.001). After separation, abruptly weaned calves spent twice as much time walking as two-stage calves on all four days observed in Trial 1 (Figure 3.5 A), and they also spent twice as much time walking during the first three days in Trial 2 (Figure 3.5 B). Peaks in

Figure 3.4. Mean (±SD) vocalization rates[1] for calves (calls/d) on each day of the experiment. Calves were either weaned in two-stages - - -●- - - (nursing deprived for 5 d [A] or 3 d [B] prior to separation), or abruptly weaned by separation —■— . Italicized *P*-values in parentheses indicate significant treatment effects within each period. Treatment effects on specific days of the experiment are shown by asterisks: $P<0.05$ (*), $P<0.01$ (**) and $P<0.001$ (***). Statistical tendencies on specific days are indicated by the actual *P*-value. [1]Call rates were calculated based on the number of calls recorded during a 2-min sampling period, taken every 5 min during daylight hours.

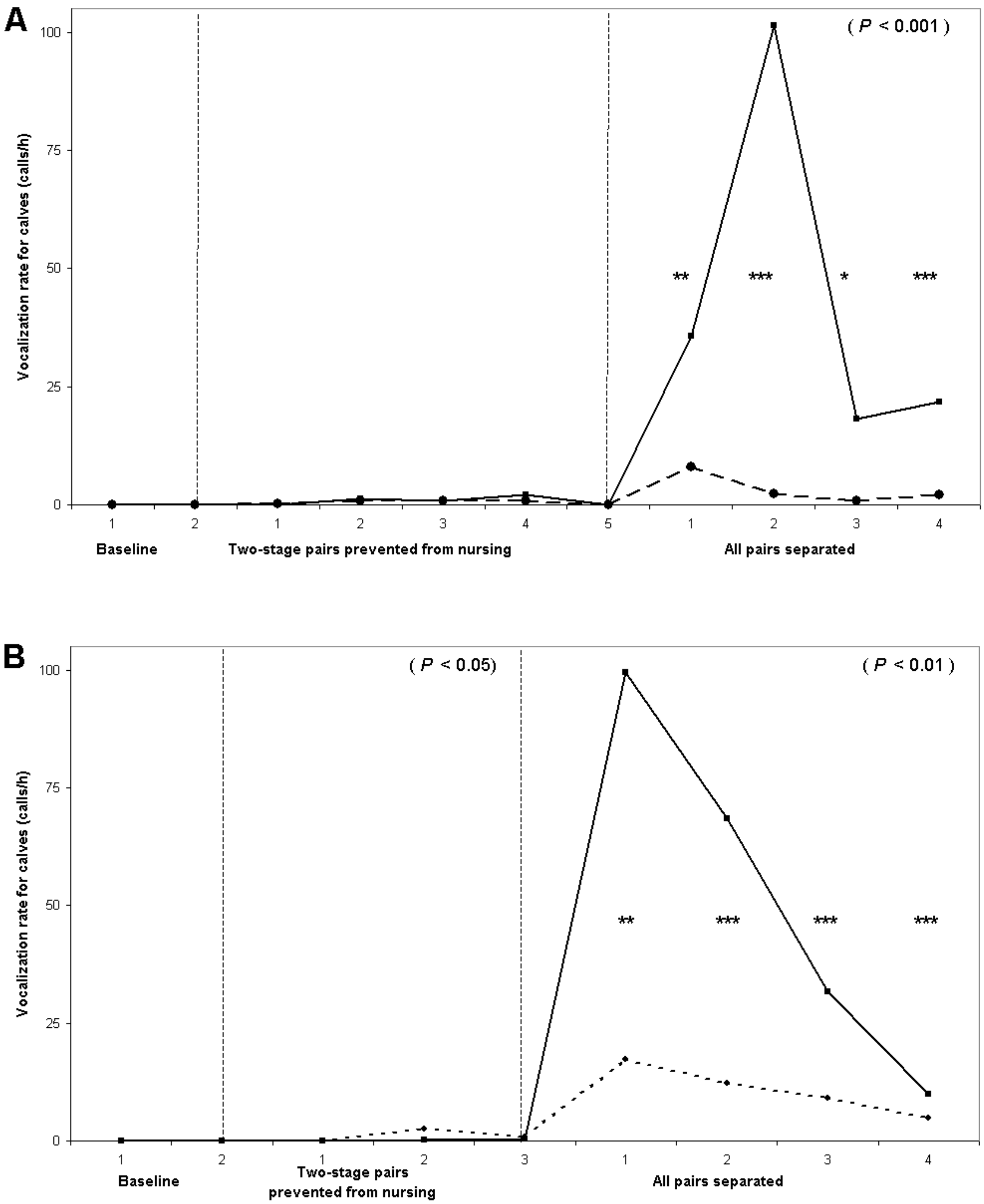
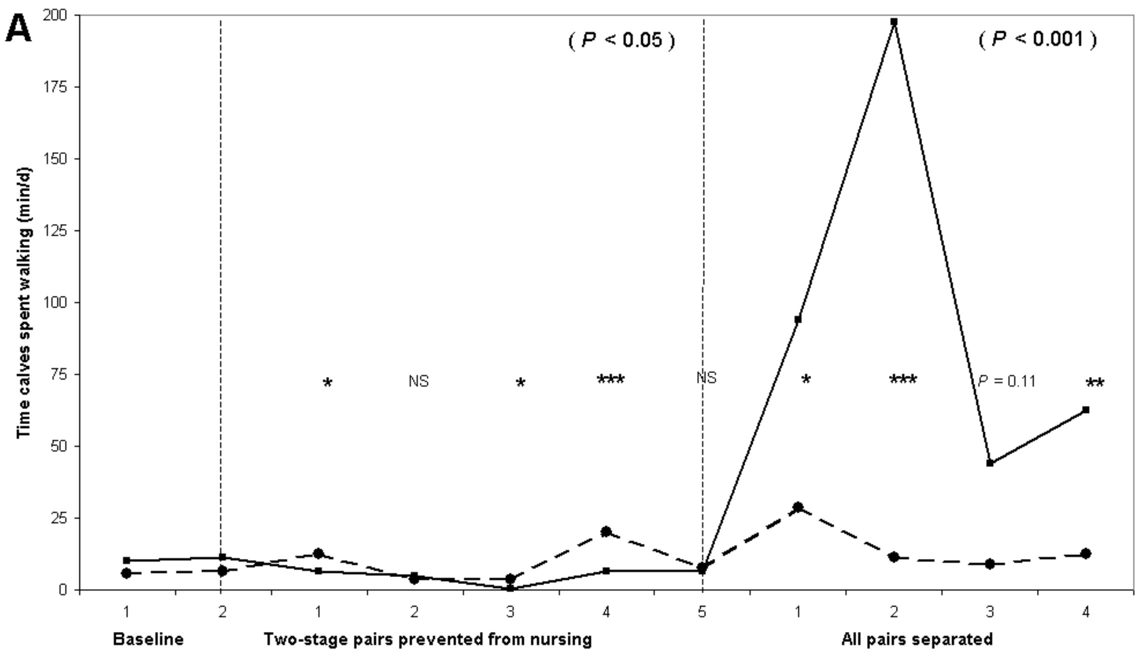
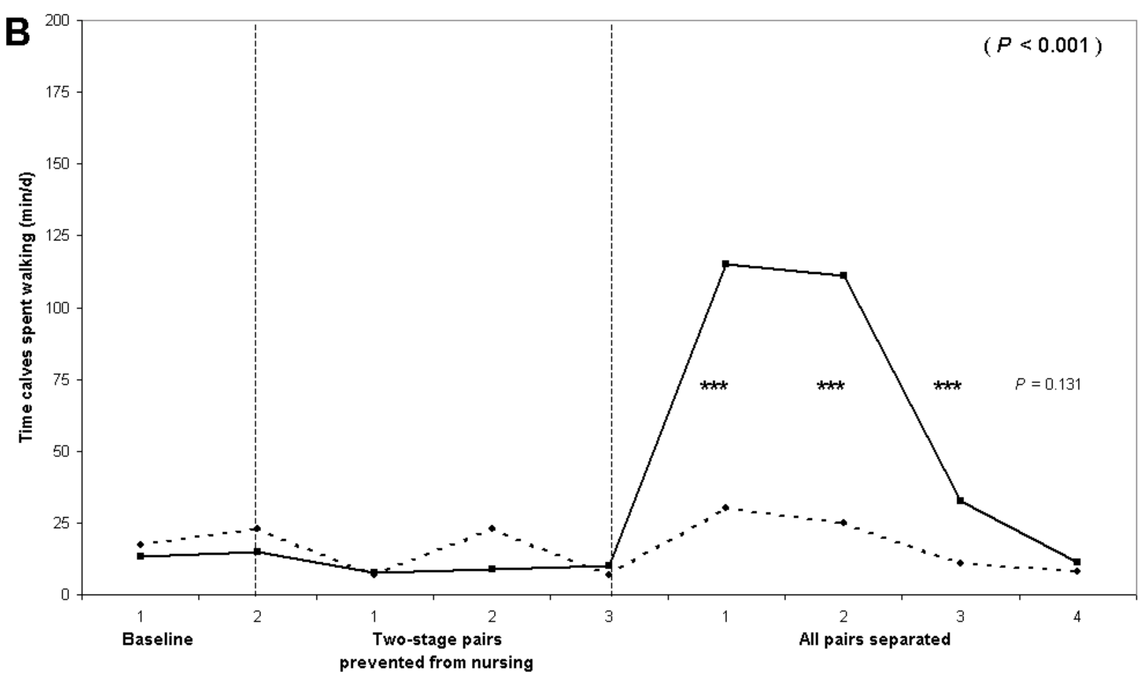

A
(P < 0.001)
Vocalization rate for calves (calls/h)
100
75
50
25
0
** *** * ***
1 2 1 2 3 4 5 1 2 3 4
Baseline
Two-stage pairs prevented from nursing
All pairs separated
B
(P < 0.05)
(P < 0.01)
Vocalization rate for calves (calls/h)
100
75
50
25
0
** *** *** ***
1 2 1 2 3 1 2 3 4
Baseline
Two-stage pairs prevented from nursing
All pairs separated

Figure 3.5. Mean (±SD) time[1] (min/d, (min per 12-h observed) calves spent walking each day of the experiment. Calves were either weaned in two stages - - -●- - - (nursing deprived for 5 d [A] or 3 d [B] prior to separation), or abruptly weaned —■— . Italicized *P*-values in parentheses indicate significant treatment effects within each period. Treatment effects on specific days of the experiment are shown by asterisks: *P*<0.05 (*), *P*<0.01 (**) and *P*<0.001 (***).


A
200
175
150
125
100
75
50
25
0
Time calves spent walking (min/d)
(P < 0.05)
(P < 0.001)
*
NS
*
***
NS
*
***
P = 0.11
**
1
2
1
2
3
4
5
1
2
3
4
Baseline
Two-stage pairs prevented from nursing
All pairs separated


B
200
175
150
125
100
75
50
25
0
Time calves spent walking (min/d)
(P < 0.001)
***
***
***
P = 0.131
1
2
1
2
3
1
2
3
4
Baseline
Two-stage pairs
prevented from nursing
All pairs separated

time spent walking by calves closely followed changes in their rate of vocalizing. Following separation, two-stage calves spent more time eating than controls (Trial 1: 43% more time, $P<0.01$; Trial 2: 26% more time eating, $P<0.001$).

In both trials control calves were more aggressive than two-stage calves (Trial 1: 9.8 vs. 2.6 acts of aggression/d, $P<0.05$; Trial 2: 5.8 vs. 2.9 acts/d, $P<0.05$).

3.3.4 Overall response to weaning

Combining the behavioural responses from Stage 1 and Stage 2, two-stage cows called less, walked less and spent more time eating, and more time lying, compared to controls. Two-stage calves called less, walked less, and were less aggressive than abruptly weaned calves.

Two-stage calves spent more time eating and more time lying in Trial 1 though there were no overall treatment differences for these variables in Trial 2.

3.5 DISCUSSION

In this study, I successfully isolated the effects of two factors normally confounded by artificial weaning; the effect of terminating nursing and the effect of separating the dam and offspring. The experimental design does not permit me to determine the relative contribution of these two factors to the overall response and these factors were only manipulated in sequential order. However, the present results show that terminating nursing and physically separating cows and calves both affect the response. Moreover, it is apparent that terminating nursing *by* imposing physical separation, the standard industry method of weaning cattle, actually exacerbates the behavioural response of both cows and calves.

It is difficult to account for treatment differences detected before any treatment was actually applied (Table 3.1, Period 1) as all pairs were randomly assigned to treatment groups. These differences may be the result of low animal numbers, which were chosen for the sake of managing detailed behaviour observations. Additionally, it would be preferable to have observations covering a longer period of time to establish baseline levels of activity behaviour.

Behaviour was less affected by weaning in two stages than abruptly weaning by separation. The reduced response was not simply due to a dilution of responses over the weaning period, but rather a lower total response over the weaning period. The effects of treatment on behaviour were still observable 4 d following separation (e.g., rate of vocalizing in Trial 1). In a previous study, employing similar observation schedules, the behaviour of calves weaned by fenceline could not be differentiated from calves weaned by distant separation four days after separation (Stookey et al., 1997). Other research has not been able to detect treatment differences four days after separation (Veissier et al., 1989a). These current results suggest that weaning in two stages has quite long-lasting effects on behaviour as treatment differences were detectable for at least an additional 24 h compared to previous weaning studies.

Veissier and le Neindre (1989) investigated the effects of artificial weaning on calves' social organization. They compared the social relationships of calves that were still nursing their dams to a group of calves also with their dams, but prevented from nursing by using cloth to cover their dams' udders. Preventing nursing in the presence of the dam was not associated with major changes in the social relationships between calves, or between calves and their dams. Cows and calves in their study were not separated as part of the experiment and behaviour recordings were designed to describe the degree of synchronicity between the different social groups. Preventing nursing had some small effects on the general activity patterns of cows and calves compared to nursing controls. However, these effects were relatively minor compared with changes resulting from weaning by separation.

Orihuela et al. (2004) weaned sheep by using cloth to cover ewes' udders and preventing nursing for 10 d prior to separation. The treatment caused ewes to vocalize more than nursing controls over the first 3 d the treatment was applied. However, ewes and lambs from these two groups did not respond differently to subsequent separation. The lack of significant results was attributed to the fact that lambs were close to their natural weaning age at the time of the experiment.

In this study, only calling by cows was significantly affected by preventing nursing (Stage 1). Cows produced 4-12 more calls/h than they had during the baseline period. It has been suggested that vocalizations may communicate information about an animal's emotional state (Watts and Stookey, 2000). Such signaling systems would be expected to evolve to indicate need (Godfray, 1995; Weary et al., 1997; Watts and Stookey, 2000). Though vocalizing by cows and calves has been associated with attempts to reunite (Watts, 2001), two-stage cows were observed vocalizing when their calves were close beside them, even as calves attempted to nurse. Though vocalizations were small in number when nursing was prevented, calls were distinctly different from those heard following separation. The calls corresponded to the low amplitude sound "Mmm" (heard when nursing was prevented) and high amplitude "Mooeh" (heard following separation) call sounds as described by Kiley (1972).

Winblad von Walter et al. (1999) noted that omitting milking caused dairy goats to alter their general daytime activity patterns of standing and lying. Although vocalizations were not reported in their study, these too might be affected by the physical sensations (e.g., pain) associated with increased intramammary pressure when regular milk-removal is withheld. The slightly increased rate of vocalizing by cows in this study might reflect their motivation to nurse. Nursing may have rewarding properties in terms of reducing intramammary pressure. Further work analyzing the acoustic characteristics and precise contexts of vocalizations when nursing is prevented could help to differentiate the significance of these vocalizations.

During Stage 1, calves wearing the antisucking device spent the same amount of time eating and drinking as controls. This result provides some evidence that the device did not greatly interfere with these behaviour patterns. The minor changes in behaviour (slight increases in the rate of vocalizing) suggest calves were not severely distressed when nursing was prevented, although it may be useful to evaluate the physiological response of calves in this situation as well.

Changes in the behaviour of two-stage calves were not completely consistent between trials 1 and 2. Preventing nursing for a longer period prior to separation (Trial 1=5 d vs. Trial 2=3 d) may be responsible for the lack of matching results. In Trial 2, calves prevented from nursing vocalized more frequently and spent less time lying and ruminating compared with controls. Another possible explanation for the difference between trials is that at least some calves in Trial 2 may not have fully received the intended treatment (i.e., no nursing while in the presence of their dam) due to recurring failure of the antisucking devices.

Napolitano et al. (2003) allowed the bond between ewes and their lambs to develop normally for the first 30 h of life and thereafter covered the ewe's udder with cloth to prevent nursing and found attachment declined. In the current study I did not test whether the reduced response of two-stage animals to separation was due to a reduced bond between the cow and calf. However, during the 4 d nursing was deprived, cows and their calves appeared to spend more time in close physical proximity to one another compared with pairs still able to nurse. This casual observation has since been confirmed (Haley et al., 2005). While one hypothesis could be that weaning in two stages somehow promotes independence, the fact that dams and their offspring were in such close physical contact when prevented from nursing, may well mean their attachment, at least initially, remained intact or possibly was even increased. Physical proximity between dam and offspring has been suggested as a possible measure of attachment (Gubernick, 1981).

The response of control cows and calves in the current study corresponds with observations by others who have also documented increased calling and walking and decreased time spent eating after separation (Veissier et al., 1989a; Stookey et al., 1997; Price et al., 2003). The same behavioural responses occurred with weaning in two stages, although less pronounced. Two-stage cows and their calves called less, walked less and spent more time eating than controls. Watts (2001), observed free ranging cows and calves that had become visually separated from one another while grazing, and found that rate of vocalizing and time spent walking were inversely related to the amount of time required for the pair to reunite. Allocating more time to these activities (e.g., vocalizing, walking) obviously affects the time available for engaging in other behaviour (e.g., eating, lying) and thus the behavioural responses to weaning should not be considered independent.

Vocalizing and walking are common behavioural responses to dam-offspring separation in a variety of species (e.g., cattle: Veissier et al., 1989a; horses: Hoffman et al., 1995; sheep: Orgeur et al., 1999; red deer: Pollard et al., 1992). These general response patterns for both cows and calves are less pronounced if separation occurs when calves are very young (e.g., 1 vs. 14 days of age; Flower and Weary, 2000). The extent to which this response may be linearly related to age for cattle is not known.

The response to weaning may be related to the level of parent-offspring conflict over the allocation of resources (see Trivers, 1974). Specifically, the response of cattle to weaning may relate to the dam's level of milk production, as milk yield has been shown to affect the natural weaning process in sheep (Arnold et al., 1979). For example, piglets that suckle the anterior teats of the sow and receive more milk would suffer more nutritional deprivation after weaning and they also perform more low-frequency "begging calls" after weaning (Mason et al., 2003).

In addition to those behaviour changes previously mentioned, I also found increased levels of aggression by calves following separation, with control calves generally being more aggressive. As the number of available feeding spaces effectively increased when cows were removed from the pen it is unlikely the aggression was caused by competition for food. Veissier and le Neindre (1989) also found an increase in agonistic interactions when calves were abruptly weaned by separation. They also reported that calves prevented from nursing were more aggressive than calves that were nursing, which was not found in this study. The increased aggression by control calves following separation could be due to the fact they spent more time walking, which could increase their number of encounters with other calves. However, many aggressive interactions were preceded by a seemingly targeted running charge of several steps. Control calves engaged in significantly more agonistic encounters and there were clear treatment differences, even though they were housed together in the same pen. This represents a very interesting difference between these two treatments and their experience at weaning.

Though treatment groups were housed together, both cows and calves were moved into new pens after separation, potentially changing their behaviour, and sensitivity to external stimuli (Done-Currie et al., 1984). Any effects of the novel environment did not overshadow the treatment effects, but such environmental effects are worthy of some consideration. Price et al. (2003) noted calves weaned by distant separation from their dams spent more time walking when weaned to pasture, where they had more space compared to a dry-lot environment.

Keeping two-stage calves with the control group may have had some effect on the their response. The increased activity of control animals may have increased responses by the two-stage calves, or vice versa. Thus treatment differences would likely have been more pronounced if the two groups had been housed separately.

At the outset, the question was raised about whether terminating nursing and physically separating pairs might contribute in different ways to the behavioural response of cattle to weaning imposed by separation. Another question put forward was whether one factor might be a more important in affecting the behavioural response to the process of artificial weaning. These two factors could not be directly compared in the present trials due to confounding day effects. An experimental design to more completely address this question would need to look at the order of eliminating these two factors. Nevertheless, the sequential order followed here greatly reduced the behavioural signs of distress. The present results suggest these two factors do produce separate effects. The simultaneous elimination of nursing and physical contact, as per standard industry practice, has a far greater effect than eliminating these two factors sequentially, one at a time. There are other examples of additive effects caused by multiple stressors in other animals (poultry: McFarlane et al., 1989; pigs: Hyun et al., 1998). If terminating the availability of milk and separating the cow and calf are regarded as two independent stressors that together cause a significant and negative synergistic effect, then two-stage weaning would seem a viable alternative to the present method of weaning practiced by the beef industry in North America.

The results of this study have demonstrated a practical alternative method for weaning cattle. Preventing nursing but allowing physical interaction between the dam and offspring facilitates their subsequent separation. Terminating nursing for just 3 to 5 days before separation seems to be adequate to greatly reduce the behavioural responses to separation by both the cow and calf.

4.0 WEANING CATTLE IN TWO STAGES: THE EFFECTS OF PREVENTING NURSING FOR 4 OR 8 DAYS ON THE RESPONSE OF COWS AND CALVES TO SUBSEQUENT PHYSICAL SEPARATION

4. 1 ABSTRACT

Weaning cattle in two stages by preventing nursing for a few days (Stage 1) before physically separating the cows and calves (Stage 2), reduces behavioural signs of distress compared with weaning abruptly, by separation. In this study I examined whether the length of time pairs are prevented from nursing (8 vs. 4 d) would affect their behavioural response to being physically separated. A total of 18 cow-calf pairs were weaned by preventing nursing for 8 d (n=6), 4 d (n=6) or 0 d (abrupt weaning control, n=6), prior to separation. Calves in the 8- and 4-d groups wore a plastic antisucking device in their nose, which prevented nursing but otherwise permitted them full contact and interaction with their dam. When prevented from nursing (Stage 1), two-stage animals called more than the control calves that were still able to nurse (two-stage treatments combined vs. controls (cows=5.1 vs. 0.6 calls/h, $P<0.01$; calves=1.5 vs. 0.1 calls/h, $P<0.001$). Preventing nursing for twice as long had no significant effects on the response of cows and calves to separation. Both two-stage treatment groups responded less to weaning than controls weaned abruptly, by separation. After separation, two-stage cows called 84% less (14.3 vs. 89.4 calls/h, $P<0.0001$), spent 60% less time walking (28.5 vs. 70.8 min/d, $P<0.001$) and 13% more time lying (165.2 vs. 146.3 min/d, $P<0.05$) than controls. After separation calves weaned in two stages called 97%

less (1.9 vs. 56.0 calls/h, $P<0.0001$), and spent 30% more time eating (267.9 vs. 206.3 min/d, $P<0.01$). Four-day two-stage calves also spent 61% less time walking (15.8 vs. 40.0 min/d; $P<0.05$) than abruptly weaned calves. These results provide further evidence that a two-stage procedure diminishes the behavioural response of cattle compared to weaning by abrupt separation. Results also suggest a period of eight days without nursing does not offer significant benefit over preventing nursing for four days prior to separation when cattle are weaned in two stages.

4.2 INTRODUCTION

Weaning cattle abruptly by separating cows and calves causes dramatic changes in their behaviour, most notably the rate of vocalizing and time spent walking increase, and time spent eating and lying decrease (Veissier and Le Neindre 1989; Veissier et al. 1989a; Chapter 3). These deviations from normal behaviour are taken as evidence of distress. Peripheral catecholamine concentrations in calves and epinephrine concentrations in their dams have been shown to increase following separation and subsequently show a significant decrease when the cows and calves are reunited (Lefcourt and Elsasser, 1995). Abrupt weaning by separation has been shown to increase cortisol levels both in the blood (McCall et al., 1987; Malinowski et al., 1990) and in the saliva of mares and foals (Moons et al., 2005). Additionally Moons et al. (2005) found heart rate was significantly higher from baseline levels following separation.

Research aimed at isolating the effects of terminating nursing from the effects of separating cows and calves on their response to weaning, spawned a two-stage weaning method that significantly reduces the behavioural signs of distress (Chapter 3). The two-stage method involves preventing nursing between pairs for a period of time (Stage 1) before the cows and calves are finally separated (Stage 2). Preventing nursing results in only a slight rise in the rate of vocalizing by cows and calves and their subsequent response to separation is significantly reduced over controls weaned abruptly, by separation. Two-stage cows and their calves called less, spent less time walking, more time eating and additionally the two-stage calves were less aggressive. Over the course of both stages (the entire weaning process), the two-stage method resulted in a lower rate of vocalizing and in cattle spending less time walking and a greater proportion of their time eating.

The period that nursing was prevented previously (Chapter 3) was 5 days. The rationale for this duration was that it served as a liberal estimate of how long cattle may take to resume normal behaviour following weaning by separation. Previous research has shown that behaviour patterns return to normal baseline levels about 96 h after abrupt weaning by separation (Veissier et al., 1989b). Thus, if the behavioural response of cows and calves was mainly due to the termination of nursing the animals should have resumed normal behaviour after 5 days. This in turn would enhance an evaluation of their subsequent response to separation.

Preventing nursing between two-stage pairs produced few behaviour changes typical of their distress response to weaning by separation, and general activity remained similar to baseline measures during that time. Although it may have appeared that separation had a greater effect on the behaviour of two-stage pairs than preventing nursing, their response to separation was still significantly lower than the response of controls. Furthermore, it could not be determined to what degree the responses of two-stage pairs was due to actual distress from separation or to what degree they may have been influenced by the behaviour of their abruptly weaned counterparts.

Preventing nursing for 3 to 5 d reduced but did not completely eliminate the response of cows and calves to separation (Chapter 3). The attachment between cows and calves may remain for some time even after weaning or after nursing is prevented. Veissier et al. (1990) found that calves weaned abruptly were still attracted to their dams for three weeks following separation, but that cows were no longer attracted to the calves. Napolitano et al. (2003) found that the ewe-lamb bond gradually declined when the ewe's udder was covered with cloth to prevent nursing.

For this study it was hypothesized that the longer pairs went without nursing the less their response would be to subsequent separation.

4.3 MATERIALS AND METHODS

All experimental procedures used in this study were approved by the University of Saskatchewan's Committee on Animal Care and Supply (UCACS Protocol #20000096) and animals were cared for according to Guidelines set by the Canadian Council on Animal Care (1993).

4.3.1 Animals

A total of 18 crossbred cow-calf pairs were used in this study. The average (mean ± SD) age of the cows was 3.2±2.5 years of age (range=2 to 10 years) and they were primarily of Charolais, Hereford and Simmental breed origins. All calves in the study were female, sired by either Charolais or Hereford bulls, and their mean age was 204±19 d when they were separated from their dams.

4.3.2 Treatments and experimental design

Previously, preventing nursing between cow-calf pairs for as few as 3 d had significant and beneficial effects on their subsequent response to separation compared with weaning abruptly by separation (Chapter 3). For the present study the behavioural response of cows and calves to separation was compared after preventing nursing for 8 d (n=6), 4 d (n=6) or 0 d (abrupt weaning control, n=6).

A B

Figure 4.1. The plastic antisucking devices worn by two-stage calves to prevent nursing (A). The tongs were flexed to expand the gap opening, allowing it to be placed in the calf's nose where it hung freely, without piercing the nasal septum (B). Cows were not observed to actively reject nursing attempts; rather, the nose flap obstructed the calf's access to the teats as the calf extended its neck and head toward the udder. Calves were able to consume hay and water while wearing the device.

Nursing was prevented by an antisucking device, worn by the calves (Figure 4.1 a) Villa Nueva S.A., Villa Maria-Cordoba, Argentina). The device was 12.0 × 7.5 cm at its widest point. The flexible plastic device was twisted to expand the gap opening, which allowed it to be placed in the calf's nose where it hung freely (Figure 4.1 b), without piercing the nasal septum. The nose-flap prevented nursing by covering the calf's mouth as the calf extended its neck and head toward the udder and obstructing access to the teats. Calves were able to consume hay and drink water while wearing the device.

Animals were initially housed in two dry-lot pens (30.5 × 27.5 m). Each pen contained nine pairs, three from each treatment group. At separation the cows were moved to new dry-lot pens of the same size with their pen mates. Calves also remained with their pen mates and they stayed behind in their home pens to avoid any possible affects of changing their physical environment (Done-Currie et al., 1984). Thus separation resulted in 4 pens with 9 animals in each (2 pens of cows, 2 pens of calves).

All calves received the same amount of handling. On days when antisucking devices were fitted, all the calves were handled. At no time did control calves wear the antisucking device or any parallel substitute. After separation, the cows and calves were kept in pens 60 m apart with 5% porosity fencing. Thus pairs were within auditory range, with perhaps some limited visual contact but no physical contact.

Animals were fed free-choice grass hay and water ad libitum for the duration of the experiment.

4.3.3 Behaviour observations

This study lasted a total of 16 days and consisted of three distinct time periods. First, baseline behaviour levels were recorded over 4 d (Period 1). Next, over 8 d two-stage pairs were prevented from nursing, either for all 8 d or over the final 4 d (Period 2, Stage 1). Lastly pairs from all treatment groups were separated and their behaviour recorded for 4 d (Period 3, Stage 2; see Figure 4.2).

Behaviour was recorded by live observation on every day of the study from 0700-1900 hours, which during the first two weeks of September, constituted the entire daylight period for our geographic location (52°7'54" N, 106°39'9" W). Cows and calves were numbered with livestock paint to facilitate individual identification. The general activity of each individual was recorded every 10 min by instantaneous scan sampling. Lying, standing, walking, eating, ruminating and any of their possible combinations (e.g., lying and ruminating) were recorded. Nursing was recorded by one-zero sampling every 5 min. Nursing was recorded to verify no pairs had already weaned naturally, but beyond the baseline this same method was used to record nursing attempts by calves wearing the antisucking device. A nursing attempt was defined by the calf's nose making contact with the udder.

After each instantaneous scan sample the number of vocalizations was recorded for every animal during 2 consecutive minutes. Any audible vocal sound that could be

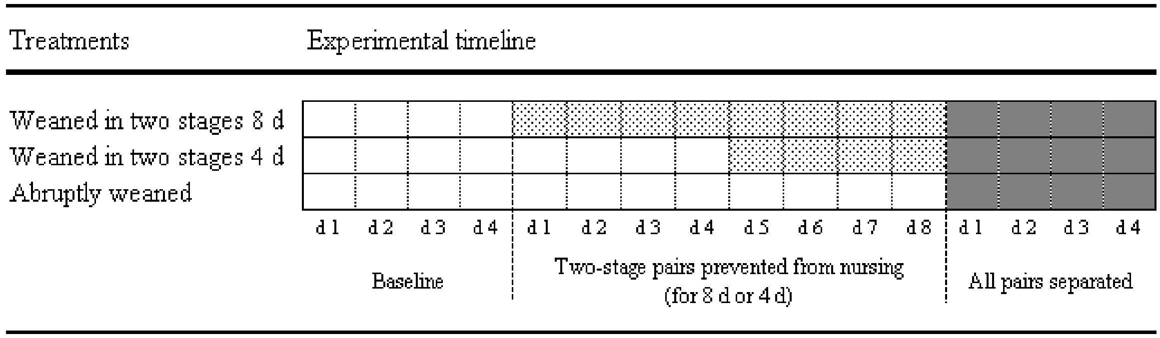


Figure 4.2. Treatments and the experimental timeline for the present study, illustrating the days when cows and calves were nursing , days when two-stage cows and calves were together, but prevented from nursing , and days when the cows and calves from all treatment groups were apart .

attributed to a specific individual was counted as a vocalization. Bursts of vocalizing were recorded by counting the number of individual short successive calls within each sequence, as distinguished by inhalations taken by the animal between each separate call (see Kiley, 1972, See-saw calls - type B, p. 193).

The number of aggressive acts was recorded continuously for 5 min of each 10-min interval. Aggression was defined as any head-butt that made physical contact with another animal and either caused that animal to move away or reciprocate contact.

Before separation, a single observer was able to record the behaviour of all the cows and calves, alternating between the two pens of animals every 5 min. Separation resulted in the formation of four pens of animals and two observers were then needed to record behaviour. Observers balanced the time they spent watching each of the four pens.

4.3.4 Statistical analysis

Data were transformed to indicate the rate of vocalizing (calls/h), the rate of aggressive interactions (acts/h) and the duration of time (min/d) that animals spent engaged in the various behavioural states recorded (e.g., lying, walking, eating, etc.). For example, vocalizations were actually recorded over a total of 144 min each day (for 2 of every 10 min, over 12 h/d). Thus the sum of vocalizations by an individual for any given day was multiplied by 5 to yield the total number of calls/12 h observed, which was then divided by 12 to yield the number of calls/h. Aggressive acts were recorded for 5 out of every 10 min and the instantaneous samples of state behaviour patterns were recorded as descriptors for the entire 10-min interval and so calculations were made accordingly.

Analyses were performed within the three specified time periods described previously. Additionally, to test whether the overall weaning response of the two-stage pairs was different from controls or simply the same response diluted over a greater number of days data was pooled and analyzed across periods 2 and 3.

All data were analyzed using a generalized estimating equations (GEE) method to account for repeated measures taken on the same cows and calves. Data were analyzed using a statistical computer software program (SAS v.8.2 for Windows (PROC GENMOD); SAS Institute, Cary, North Carolina, USA, 1997). Model specifications included a normal distribution, identity link function, repeated statement with subject equal to calf identification, and an AR(1) correlation structure. Variables remaining in the final multivariable model at $P<0.05$, based on the robust empirical standard errors produced by the GEE analysis, were considered statistically significant.

4.4 RESULTS

Complete behaviour results for this study are presented in Table 4.1 (A: cows, B: calves).

Table 4.1. Behaviour means (± SE) for cows (A) and calves (B) from two-stage 8 d, two-stage 4 d, and abrupt weaning treatment groups. The rate of vocalizing is expressed in calls/h and other behaviour variables are expressed in min/d (min per 12-h observed). Analyses were performed within specific time periods: 1) a 4-d baseline period when all pairs were nursing, 2) the 8-d period when two-stage pairs were prevented from nursing for either 8 or 4 d, and 3) the 4-d period after cows and calves had been separated. Within each row, means with different letters differ by at least $P < 0.05$.

A Period Behaviour	COWS Two stage weaning 8 d	 Two stage weaning 4 d	 Abrupt weaning
Period 1 (baseline)			
Vocalizing	0.7 ± 0.2	1.9 ± 0.8	1.1 ± 0.3
Walking	10.9 ± 2.2 a	16.7 ± 2.3 b	16.0 ± 1.8 b
Eating	281.3 ± 11.6	265.4 ± 9.2	250.8 ± 7.4
Lying	195.2 ± 13.6	213.3 ± 15.6	248.0 ± 16.2
Ruminating	179.1 ± 9.4	180.4 ± 9.2	175.6 ± 8.4
Period 2 (two-stage pairs prevented from nursing for 8 d or 4 d)			
Vocalizing	6.5 ± 1.7 a	7.4 ± 1.6 a	2.4 ± 0.3 b
Walking	14.8 ± 1.9 a	15.4 ± 1.7 a	8.5 ± 1.1 b
Eating	246.3 ± 7.1	260.2 ± 8.5	258.5 ± 6.4
Lying	199.0 ± 9.6	198.1 ± 11.4	225.6 ± 11.0
Ruminating	151.5 ± 8.1	165.4 ± 8.3	165.6 ± 7.1
Period 3 (all pairs separated)			
Vocalizing	12.2 ± 5.0 a	19.0 ± 5.2 a	90.6 ± 17.6 b
Walking	24.2 ± 5.2 a	32.9 ± 5.6 a	70.8 ± 11.9 b
Eating	267.1 ± 12.5 a	255.0 ± 14.4 a b	223.8 ± 10.9 b
Lying	160.0 ± 16.6	170.4 ± 18.4	146.3 ± 21.0
Ruminating	153.8 ± 13.5	142.1 ± 13.6	145.0 ± 15.2

Means (±SEM) on the same line with different letters differ by at least $P < 0.05$

Period Behaviour	CALVES Two stage weaning 8 d		Two stage weaning 4 d		Abrupt weaning	
B						
Period 1 (baseline)						
Vocalizing	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
Walking	14.3 ± 2.7		18.8 ± 2.8		15.6 ± 2.2	
Eating	200.9 ± 9.2		201.7 ± 11.7		204.0 ± 8.0	
Lying	327.0 ± 10.9		335.8 ± 11.3		342.4 ± 10.7	
Ruminating	174.3 ± 8.2		180.8 ± 8.6		164.4 ± 9.3	
Period 2 (two-stage pairs prevented from nursing for 8 d or 4 d)						
Vocalizing	1.9 ± 0.5	a	1.1 ± 0.4	a	0.2 ± 0.1	b
Walking	12.3 ± 1.6		10.4 ± 1.5		9.4 ± 1.3	
Eating	215.0 ± 9.9		214.6 ± 9.2		215.0 ± 9.8	
Lying	295.0 ± 9.2		297.5 ± 9.1		312.7 ± 7.6	
Ruminating	164.2 ± 7.3		142.9 ± 6.7		146.5 ± 6.8	
Period 3 (all pairs separated)						
Vocalizing	1.8 ± 0.4	a	2.0 ± 0.6	a	56.0 ± 9.6	b
Walking	15.8 ± 2.9	a	15.8 ± 2.8	a	40.0 ± 9.0	b
Eating	265.4 ± 12.2	a	270.4 ± 12.7	a	206.3 ± 12.3	b
Lying	238.3 ± 12.3		249.2 ± 12.6		230.0 ± 20.3	
Ruminating	132.1 ± 11.1		120.8 ± 11.2		130.4 ± 11.9	

Means (±SEM) on the same line with different letters differ by at least $P < 0.05$

4.4.1 Period 1 (baseline, 4 d)

The behaviour of treatment groups was similar during the baseline period. The sole difference was that cows destined to receive the two-stage 8 d treatment spent less time walking than the other two groups. The rate of vocalizing was very low during this period that pairs were still nursing (means, cows=1.2 calls/h; calves=0.03 calls/h).

4.4.2 Period 2 (Stage 1, 8 d)

Cows and calves from both two-stage treatment groups responded similarly when nursing was prevented. The rate of vocalizing increased for both the cows and their calves compared to their control counterparts that were still able to nurse (cows: $P<0.05$; calves: $P<0.05$). On average over the 8-d period, two-stage cows produced 4.6 calls/h more than control cows. Two-stage calves averaged 1.5 calls/h during this period, while control calves averaged 0.2 calls/h. Additionally, two-stage cows spent about twice as much time walking as cows that were nursing their calves (15.1 vs. 8.5 min/d, $P<0.05$).

4.4.3 Period 3 (Stage 2, 4 d)

The behavioural response of two-stage cows and calves to separation was similar, whether nursing had been prevented for 8 d or 4 d prior to separation. The only difference detected was that two-stage cows deprived of nursing for 8 d, spent more time eating following separation compared to abruptly weaned cows (43.3 more min/d;

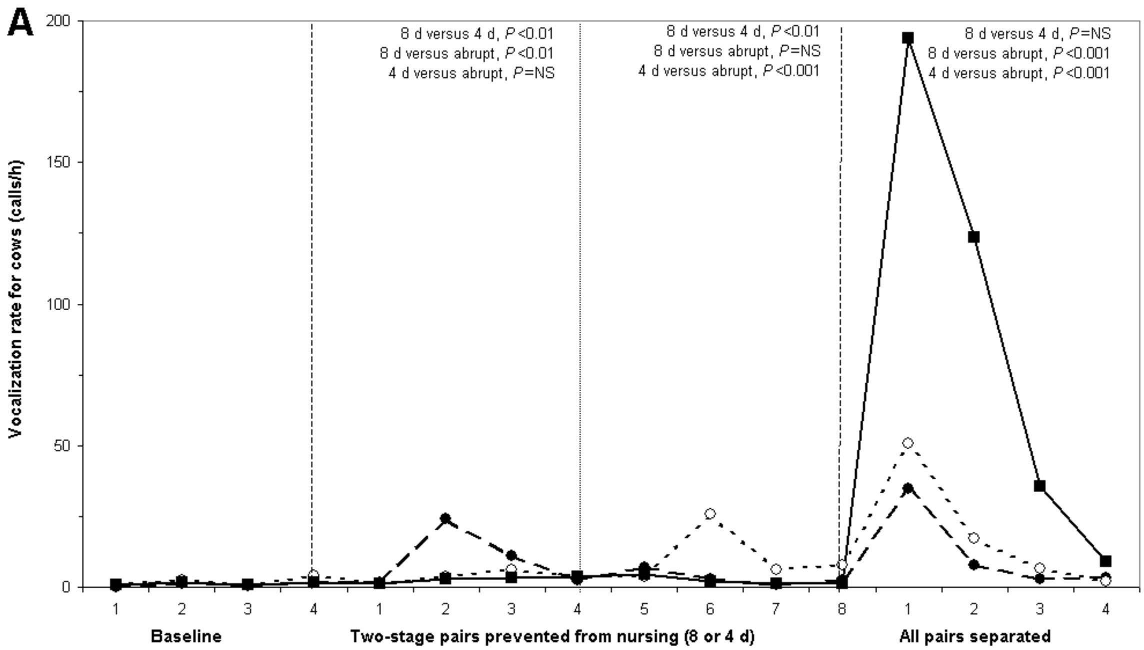
$P<0.05$) As there were no other differences between two-stage treatment groups, the remaining results for this study represent the combined average response variables for both two-stage treatment groups.

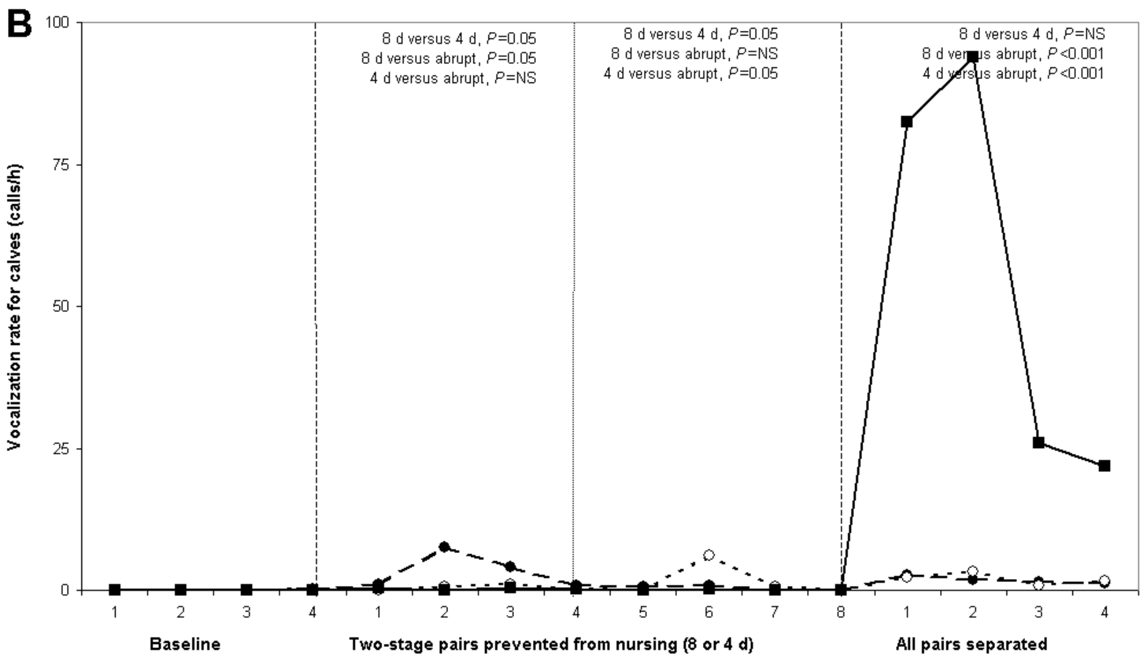
The behavioural response to two-stage weaning was significantly different from the response to abrupt weaning, for both the cows and the calves. Cows weaned in two stages called 83% less than abruptly weaned cows ($P<0.05$) while their calves called 88% less than the controls ($P<0.05$). The average rate of vocalizing by abruptly weaned cows, over the four days observed following separation, was 90 calls/h, while their calves called at a rate of 56 calls/h. The effects of treatment on calling on individual days after separation are presented in Figure 4.3 (A=cows, B=calves). Walking behaviour was also affected by weaning in two stages. Two-stage cows spent 60% less time walking ($P<0.05$) and their calves spent 61% less time walking ($P<0.05$) compared with their control group counterparts. Two-stage calves also spent on average 30% more time eating over the four days following separation compared to those calves weaned abruptly by separation ($P<0.05$).

4.4.4 Overall response to weaning

In this study, weaning in two stages occurred over a total of either 12 d or 8 d, compared to abrupt weaning, for which the response was concentrated over the 4 d after separation. The behaviour of the three treatment groups was compared across Stages 1

Figure 4.3. Mean (±SD) vocalization rates for cows [A] and calves [B] on each day of the experiment. Cows and calves were either weaned in two stages with nursing prevented for 8 d (– – ● – –), or 4 d (- - - ○ - - -) before separation, or weaned abruptly, by separation (—■—). Data from the baseline period represent absolute values (calls/h)[1]. Data from the treatment and separation periods represent the daily differences from the mean baseline value, for respective treatment groups. *P*-values indicate significant treatment effects within each period at the level *P*<0.001 (***).[1] All call rates are calculated based on the number of calls recorded during a 2-min sampling period, taken every 10 min over 12 h of observation.


A
Vocalization rate for cows (calls/h)
8 d versus 4 d, P<0.01
8 d versus abrupt, P<0.01
4 d versus abrupt, P=NS
8 d versus 4 d, P<0.01
8 d versus abrupt, P=NS
4 d versus abrupt, P<0.001
8 d versus 4 d, P=NS
8 d versus abrupt, P<0.001
4 d versus abrupt, P<0.001
Baseline
Two-stage pairs prevented from nursing (8 or 4 d)
All pairs separated


B
Vocalization rate for calves (calls/h)
8 d versus 4 d, P=0.05
8 d versus abrupt, P=0.05
4 d versus abrupt, P=NS
8 d versus 4 d, P=0.05
8 d versus abrupt, P=NS
4 d versus abrupt, P=0.05
8 d versus 4 d, P=NS
8 d versus abrupt, P<0.001
4 d versus abrupt, P<0.001
Baseline
Two-stage pairs prevented from nursing (8 or 4 d)
All pairs separated

and 2 to assess whether two-stage animals were in fact showing the same response to weaning as controls, only diluted over time. Again, there were no differences between the two, two-stage treatments. Overall, two-stage cows called less ($P<0.05$) and walked less ($P<0.05$) than abruptly weaned cows. Two-stage calves vocalized less than abruptly weaned calves ($P<0.05$).

4.5 DISCUSSION

Consistent with previous results (Chapter 3) this study found weaning in two stages significantly reduced the response of cows and calves to separation compared with abrupt weaning, by separation. Previously, preventing nursing for 4 d resulted in an 80% reduction in walking by calves and a 70% and 85% reduction in calling by cows and calves respectively, over abruptly weaned controls. In this study it was hypothesized that pairs prevented from nursing for 8 d would respond less to separation compared with pairs prevented from nursing for 4 d. However, two-stage cows and calves did not differ in their response to separation whether they were prevented from nursing for 8 d or 4 d.

While sufficient to distinguish between two-stage and abrupt weaning methods, the quite modest sample size of six cows and six calves per treatment may not have been sufficient for detecting differences between the two-stage weaning treatments. Based on numerical means, cows prevented from nursing for 8 d did appear to have lower responses to separation than cows prevented from nursing for 4 d, though these were not significant. No similar pattern could be seen among the behavioural response variables for two-stage calves.

Though general activity variables are often used to measure the response of cows and calves to weaning, more subtle behaviour changes may have been able to distinguish between the two-stage weaning treatments. The hypothesis was based on the notion that preventing nursing would result in dissolution of the dam-offspring bond as has been shown to occur with sheep (Napolitano et al., 2003). In the present study dissolution of the dam-offspring bond was assessed by their response to separation, which did not detect any difference between the two-stage treatments. Other variables may have been more revealing about possible changes in the dam-offspring bond. For example spatial proximity of the dam and offspring is often used as a measure of attachment (Veissier et al., 1990a). However, this is only one of multiple behaviour criteria that have been proposed to assess or even establish an attachment (Gubernick, 1981).

In this study, no specific experimental tests were carried out, which might have helped to quantify the strength of the dam-offspring bond at the time of separation. As an example, under experimental conditions Veissier et al. (1990b) used an experimental approach to assess whether offspring showed any preference to be near their dams over another familiar cow following abrupt weaning by separation. Calves still preferred to be near their dam, even after 21 d of separation. At that same point in time, cows actively rejected nursing attempts by their calves, which could be interpreted as a sign that cows were less attached to their calves than vice versa. This result might be interpreted as evidence that calves remain attached to their dams after weaning in which case they might be expected to react to being separated from them for quite some time after nursing is prevented. Preventing nursing for 8 d may not have been sufficient to further affect their general activity level. A subsequent investigation has shown the behavioural response of 185-day-old beef calves to weaning in two stages was no different whether they were prevented from nursing for 3 d or 14 d prior to separation (Haley et al., 2005).

Although Napolitano et al. (2003) found that the dam-offspring attachment gradually declined after the ewe's udder was covered with cloth to prevent nursing, this treatment was imposed within a few days of the offspring being born. In the present study the cows and calves had been together and nursing for several months, thus their attachment may have been stronger and take longer to dissolve.

The present results show the beneficial effects of two-stage weaning are gained sometime during the first 4 d that nursing was prevented. Doubling the duration of Stage 1 to 8 d did not further diminish their response to separation. The benefit gained by reducing nursing prior to separation does not appear to be linear in its relation to the amount of time that nursing is prevented.

In this study, the physical environment of the calves and the diet of all animals remained were kept constant throughout to ensure the behavioural responses were primarily due to the effects of separating the dam and offspring. Still, other factors could have influenced their response to separation and masked any potential added benefit of preventing nursing for a longer period. These factors could have included the general effects of fracturing the social group or possible influence of the distress being exhibited by pairs abruptly weaned, as all treatments were present in each pen. It may also be simply that social bonds between a cow and her calf will always result in some observable response to separation, whenever it is imposed. Cows and their calves are reported to have close associations when they are kept together beyond weaning (Veisser et al., 1990a). Furthermore, the numbers used in this investigation were quite modest even though it enabled very detailed observations. It may not be possible to completely eliminate the response of cows and calves to separation under the present experimental design.

The behavioural response of cows and calves to separation has always been assumed to reflect the strength of the bond between cows and calves. The attachment between cows and calves is the reason given for their strong reaction to abrupt weaning, even at 9 months of age (Veissier et al., 1989b; Vessier et al., 1990b). However, two-stage weaning suggests the behavioural response of cows and calves to abrupt weaning by separation is likely an exaggeration caused by the simultaneous termination of nursing and physical contact between the cow and calf. In that way, the response to abrupt weaning may be considered something of an aberration caused by the synergistic effects of multiple stressors. One stressor might be the inability to locate the missing partner and another distinct stressor might be their frustrated motivation to nurse. As reaffirmed in the present study, sequentially imposing these two restrictions, even in short succession greatly reduces the behavioural response of cows and calves to separation.

It has been pointed out previously that the lack of an overt response to weaning may not necessarily mean that calves are no longer attached to their dams. Veissier and le Neindre (1989) weaned a group of 8-month-old calves by using cloth to cover their dam's udders. They noted no significant behaviour changes when nursing was prevented and concluded by their observations that preventing suckling did not alter the relationship between the cows and their calves, suggesting the dam-offspring bond remained intact. In their study on the effect of weaning on calves' social organization, Veissier and le Neindre (1989) did not separate the cows and calves as done in the two-stage weaning process. The response of two-stage pairs to separation may be the isolated effect of being unable to locate their partner, but now without the exacerbating effects of, potentially, frustrated motivation to nurse. It might also be that the response of two-stage animals to separation may be caused in part by stimulation from the increased activity of the abruptly weaned animals as treatment groups were housed together. Separate housing for the various treatments might have altered the present results.

It is unclear at what point in time during the 4-d period of deprived nursing the actual treatment effects are achieved. It is also unclear whether, during this period, there is any relationship between the time since the last nursing and the response to separation, linear or otherwise. This would make an interesting subject for further study, which could conceivably alter our understanding of what is known about the bond between dam and offspring.

The data from this study provide further evidence that the major behavioural benefits of weaning in two stages, in terms of reducing the response of cows and calves to separation, can be achieved after denying nursing for as few as 3 or 4 d (Chapter 3). The greatest benefits gained by preventing nursing prior to separation were achieved over the first few days that nursing is prohibited. For calves in this study (roughly 200 days of age) lengthening the duration that nursing is prevented to 8 d appears to be of no obvious benefit when evaluated by behavioural measures.

5.0 THE EFFECTS OF WEANING BEEF CALVES IN TWO STAGES ON THEIR BEHAVIOUR AND GROWTH RATE

5.1 ABSTRACT

A total of 392 cow-calf pairs were used in four trials to explore possible advantages of weaning beef calves in two stages compared to the traditional method of weaning by abrupt separation. In the two-stage treatment, calves were prevented from nursing their dam for a period (stage one) prior to their separation (stage two). Control calves nursed from their dams until they were separated. Calf weights and behaviour were recorded before and after the separation of cows and calves. Following separation, calves weaned in two stages vocalized 96.6% less (P=0.001), spent 78.9% less time walking (P=0.001), spent 23.0% more time eating (=0.001) and 24.1% more time resting (P=0.001) than control calves. Compared to controls, two-stage calves had lower (P<0.001) ADG when nursing was deprived (Stage 1) but greater (P<0.001) ADG during the 7 d following separation. In Trial 3, calves weaned by the two-stage method had greater (P=0.05) growth rates than control calves for 7 wks after separation, but no treatment effects on ADG were detected (P>0.38) in Trials 1 and 2. Over the entire study period (before and after separation), ADG was similar (P>0.10) for both treatments. In Trial 4, calves weaned in two stages walked 1.3 km/d more (P<0.05) during the 4-d period when nursing was prevented (stage one) and 5.8 km/d less (P<0.05) during the 4-d period after separation than controls. Differences between treatments were the greatest on the 2 d following separation. On the first day after

separation, two-stage calves walked 5.2 ± 0.5 km/d, while control calves walked 16.7 ± 3.1 km/d. Calves weaned in two stages were less distressed than calves weaned by the traditional method of abrupt separation based on behavioural data, but overall calf ADG was similar for both methods in this study. The nutritional management of two-stage calves when they are prevented from nursing should be evaluated in future research. For example, the use of creep feeding may be important to sustain gains when milk is denied and pasture conditions are poor.

5.2 INTRODUCTION

Most beef cattle are weaned by the abrupt separation of cows and calves. Behavioural responses to this event are predictable and remain detectable for several days after separation. Cows and calves vocalize repeatedly and spend more time walking, while spending less time eating and resting (Veissier and le Neindre, 1989). These deviations from normal behaviour provide evidence that the traditional method of weaning by separation has a negative impact on the well-being of beef cattle.

Though often recommended (e.g., Neumann, 1977), separating cows and calves by the greatest distance possible does not diminish their response to traditional weaning. On the contrary, providing fenceline contact for cows and calves by separating them into adjacent pens or pastures, where they can see and hear one another, reduces vocalizing and time spent walking, increases time spent eating (Stookey et al., 1997), and improves calf ADG (Price et al., 2003).

Recently, a new method of weaning cattle in two stages has been discovered, which may reduce behavioural disruption to calves more than providing fenceline contact (Chapter 6). Preventing nursing between cow-calf pairs (stage one) prior to separation of the dam and offspring (stage two) appears to reduce the degree of behaviour changes compared to imposing both restrictions simultaneously.

The objective of this study was to further contrast the behavioural responses of calves weaned in two stages and calves weaned by abrupt separation, and to explore possible performance benefits by assessing ADG of calves weaned by these two methods. One of four trials evaluated two-stage weaning when nursing was prevented for long (14 d) and short (3 d) periods. Calves are often vaccinated at least 2 wk before weaning to reduce the possibility of respiratory diseases (Pritchard and Mendez, 1990). To minimize handling, the two-stage procedure could be initiated by fitting calves with nose-flaps when they are vaccinated before weaning.

5.3 MATERIALS AND METHODS

5.3.1 General

In accordance with the Canadian Council on Animal Care Guidelines for the Use of Animals in Research (1993), experimental procedures used in the trials described here were approved by the Committee on Animal Care and Supply at the University of

Saskatchewan (UCACS Protocol #20000096) and by the Institutional Animal Care and Use Committee at Montana State University (IACUC Protocol #1055).

In all four trials of this study, two-stage weaning was compared to a control, which was the traditional weaning practice of abruptly separating calves from their dams without other management. Calves weaned in two stages were prevented from nursing their dams for a period (Stage 1) prior to separation (Stage 2). Nursing was prevented by fitting calves with an antisucking device made of flexible plastic (Villa Nueva S.A., Villa Maria-Cordoba, Argentina; Figure 4.1). The nose-flap device (12.0 × 7.5 cm) acted as a physical barrier, which prevented calves from getting a teat into their mouth but did not interfere with grazing, eating, or drinking. Control pairs nursed until they were separated. After separation, cows and calves from all treatments were completely isolated from each other, prohibiting visual contact or vocal communication.

5.3.2 Trial 1

In this trial, two-stage calves were fitted with the antisucking device for 14 d (long two-stage treatment, n=58) or 3 d (short two-stage treatment, n=58) prior to separation, and compared to control calves (n=74). In total, 190 cow-calf pairs were used in the study, but only 116 antisucking devices were available. Cows and their calves were randomly assigned to treatment groups.

This trial was conducted at Montana State University's Northern Agricultural Research Center in Havre, MT, USA. All cow-calf pairs grazed a 421 ha pasture prior to separation, with mineral supplement and water available *ad libitum.* On the day of separation, the mean (± SD) age of calves was 187 ± 13 d (range=159 to 209 d).

5.3.2.1 Behaviour

Previous anecdotal observations suggested that when nursing is prevented between two-stage pairs, that the cow and calf may spend their time in closer physical proximity to one another compared to pairs still able to nurse. This was tested on foothill rangeland pastures on the 2 d immediately prior to the separation of cows and calves. Starting at sunrise (0630), cattle were observed within the 421 ha pasture by three observers on horseback. The purpose of the observations was to attempt a scan sample of the 190 cow-calf pairs in the pasture. During the 1.5 h/d observation periods, data was recorded for 56% of the animals. The percentages observed from each treatment group were approximately equal (long two-stage=57%, short two-stage=59%, control=53%). Binoculars allowed animals to be identified at a distance by their ear tag numbers. Observers used herd lists to identify cow-calf pairs. After noting the time and confirming ear tag numbers, the distance between the cow and her calf was estimated and scored using two categories: nearby (≤ 10 m) and distant (> 10 m).

Nose-flaps were removed from two-stage calves on the day pairs were separated, and all calves were weighed and then transported by truck for approximately 1 h to another farm where they were unloaded and left overnight. The following morning, balancing for equal numbers of males and females, 30 randomly-selected calves from each treatment were removed from the larger group and put in experimental dry-lot pens (5 × 10 m, with 5 m feeding space). A total of 15 pens were used, each containing six calves (5 pens/treatment). Each pen had grass hay and water available *ad libitum*. Remaining calves were housed together in two adjoined corrals (30 × 45 m with 25 m feeding space), but away from the experimental pens.

Observations of calf behaviour in the experimental pens started roughly 24 h after pairs had been separated. Calves were observed for 8 h from 1100 to 1900 on the first day of observation, and on the following day (the third day of separation) calves were observed for 12 h, from 0700 to 1900.

Instantaneous scan sampling was used at 10-min intervals to record the number of calves in each pen that were lying, standing, walking, eating and ruminating. Activities were not all mutually exclusive. For 2 min during each interval, the total numbers of vocalizations coming from each of three pens (one pen/treatment) were counted. All pens were also sampled an equal number of times for vocalizations, on a rotating basis (3 pens/10-min interval). Any audible vocal sound that could be attributed to a specific calf was counted as a vocalization. To avoid any potential bias, observers were blind to the assignment of treatments to pens.

5.3.2.2 Growth rate

All calves in this study were weighed 14 d and 3 d prior to separation, which corresponded to when calves from the two-stage treatment groups were fitted with nose-flaps. Calves were also weighed on the day of separation and then 8, 23 and 44 d later. For 4 d after separation, calves were kept in the pens described above and fed grass hay. All calves were then moved to a pasture that had been previously hayed. Calves grazed on the regrowth, primarily grasses, during the period from 5 to 44 d following separation.

5.3.3 Trial 2 and 3

Two additional trials were completed to compare the growth rates of calves weaned in two stages to control calves. In both trials, two-stage calves were deprived of nursing for 5 d before separation.

Trial 2 was conducted at the Western Beef Development Centre, Termuende Research Farm, Lanigan, Saskatchewan, Canada. A total of 100 calves aged 189 ± 10 d (range=158 to 214 d) at separation were weaned for this trial (two-stage, n=50; control, n=50). Calves were randomly assigned to treatment with an equal number of females and castrated males in each treatment. After separation, calves were grouped as a pen of heifers and a pen of steers. Thus, both treatment groups were managed under the same environmental conditions and feeding regimes.

Trial 3 was carried out at the University of Saskatchewan, Goodale Research Farm, Floral, Saskatchewan, Canada. A total of 52 heifer calves were weaned (two-stage, n=26; control, n=26). At separation, calves averaged 181 ± 13.7 d of age (range=137 to 201 d). Following separation, an equal number of calves from each treatment were randomly assigned to one of two pens (30.5 × 27.5 m).

5.3.3.1 Growth rate

All calves in Trial 2 and Trial 3 were weighed 5 d prior to separation, when the two-stage calves were fitted with nose-flaps. Calves were then weighed on the day of separation, and 7, 28 and 56 d after separation.

5.3.4 Trial 4

The final trial of this series was carried out at a farm near Delisle, Saskatchewan to investigate a methodology for quantifying the walking behaviour of calves at weaning time. Fifty cow-calf pairs were weaned, with an equal number of subjects randomly assigned to two-stage and control treatments. Nursing by two-stage pairs was prevented for 4 d prior to separation. Pairs were kept in a 20-ha pasture before separation. Following separation, the 50 calves were housed together in a dry-lot pen measuring 27.4 × 48.8 m with water and grass hay available *ad libitum*.

5.3.4.1 Behaviour

A subset of five randomly-selected calves from each treatment group wore a pedometer, which was securely housed in a protective plastic casing and attached the calf's front left leg with a Velcro strap (Figure 5.1; HJ-104, Omron Healthcare, Inc., Vernon Hills, Illinois). To collect baseline information about walking behaviour , the pedometers were attached 3 d prior to preventing two-stage calves from nursing. Pedometers also recorded the number of steps taken during the 4 d that two-stage calves were prevented from nursing, and for 4 d following the separation of cows and calves. The HJ-104 model featured a 7-d memory, which logged the number of steps taken, by 24-h periods. The pedometers were designed for human use and though not validated for use on cattle, precautionary measures were taken to ensure pedometers stayed in a vertical position while attached to the legs of calves in a manner similar to their intended use in humans. Each time calves were handled, the number of steps recorded by the pedometers was noted and the devices were then reset.

5.4 STATATISTICAL ANALYSIS

5.4.1 Trial 1

Data collected about the proximity of cows to their calves were first examined to ensure values for individual cows were not recorded more than once on any given day. In cases of duplicate observations of the same cow-calf pair during an observation period, only

A B

Figure 5.1. Photograph showing a pedometer and protective casing that was used in Trial 4 to continuously record the number of steps taken (A). Photograph of casing strapped to the leg of a calf (B).

the earliest observation from that day was used for analysis. Data were analyzed by using Chi-square contingency tables (Lehner, 1996). Separate 2 × 3 contingency tables (proximity category × treatment) were completed for each day of observation so that the analyses did not include repeated measures.

All 20 h of calf behaviour observed in the experimental pens were analyzed together. Total frequency counts for each behaviour variable were tallied for each pen, as were the total number of individual calf observations (calves in each pen × total number of intervals observed). All data were analyzed using a generalized estimating equations (GEE) method to account for repeated measures within pen using PROC GENMOD (SAS, 1997). Model specifications included a binomial distribution, logit link function, repeated statement with subject equal to pen number, and an AR(1) correlation structure. Variables remaining in the final multivariable model at $P<0.05$, based on the robust empirical standard errors produced by the GEE analysis, were considered statistically significant. Since vocalizations were recorded as count data they were analyzed with a Poisson distribution and log link function.

For the purpose of visualizing these data, results are presented as the percentage of observations (percent of the 20-h observed time) that individual animals spent performing each activity. Vocalizations are presented as the number of calls/h for each calf, which was estimated for individuals within each pen based on results from the interval sampling of that pen by the methods described previously.

The initial calf weights in Trial 1 collected prior to experimental manipulations were similar ($P > 0.10$) among the three treatment groups based on analysis of variance. The growth rate (ADG) of calves from all three weaning treatments was then compared during the 14 d prior to separation, during the first 7, 23 and 44 d after separation, and finally over the entire 58-d period from 14 d before, to 44 d after the separation of cows and calves. In addition, the ADG of calves was also evaluated from the time nursing ended (d 0 for controls, d -3 for short two-stage weaning and d -14 for long two-stage weaning) until the end of the study period (d 44). Analysis of ADG during each period of interest was performed separately using PROC GLM (SAS, 1997) incorporating treatment, sex and age of the calf as main factors in the final model. Interactions were evaluated, but they were not important ($P > 0.10$) and were excluded from the final model. One degree of freedom orthogonal contrasts were used to compare the two-stage treatments to controls and to compare the two-stage treatments to each other.

5.4.2 Trial 2 and 3

Growth rates from Trial 2 and Trial 3 were analyzed in the same way as Trial 1. Average daily gain was compared during the 5 d before separation (the period when two-stage calves were prevented from nursing), during the first 7 and 28 d after separation, and then over the entire 33 d period from installation of the nose-flaps to 28 d after cows and calves were separated. Calf ADG was also compared from the time that nursing ended (d –5 for two-stage calves, d 0 for controls) until the end of the study period (d 28). The model used for evaluating ADG in Trial 2 included treatment, sex and calf age. Sex was not included in Trial 3 because only heifers were used. Data from trials 2 and 3 were combined and analyzed with a model containing study site (Termuende and Goodale), calf age, and weaning treatment (two-stage and control).

5.4.3 Trial 4

The number of steps taken by calves was analyzed for 4 distinct time periods: the baseline period (3 d) when all pairs were nursing, the 4 d prior to separation (two-stage calves prevented from nursing), the 4 d after separation and the 8 d period from placement of the nose-flaps until 4 d after separation. Steps were analyzed using the GEE method to account for repeated measures taken on the same calf (SAS, 1997). Model specifications included a normal distribution, identity link function, repeated statement with subject equal to calf identification, and an AR(1) correlation structure. Variables remaining in the final multivariable model at the $P<0.05$ level, based on the robust empirical standard errors produced by the GEE analysis, were considered statistically significant.

The effects of treatment and day on the number of steps walked by calves were analyzed during the same four time periods listed above. Associations between both day and treatment for the number of steps taken were first examined alone. When both of these factors were significant then treatment and day were examined together with the treatment × day interaction term. If the interaction term was significant, then treatment effects were examined on individual days. The control group was always used as the reference group.

5.5 RESULTS

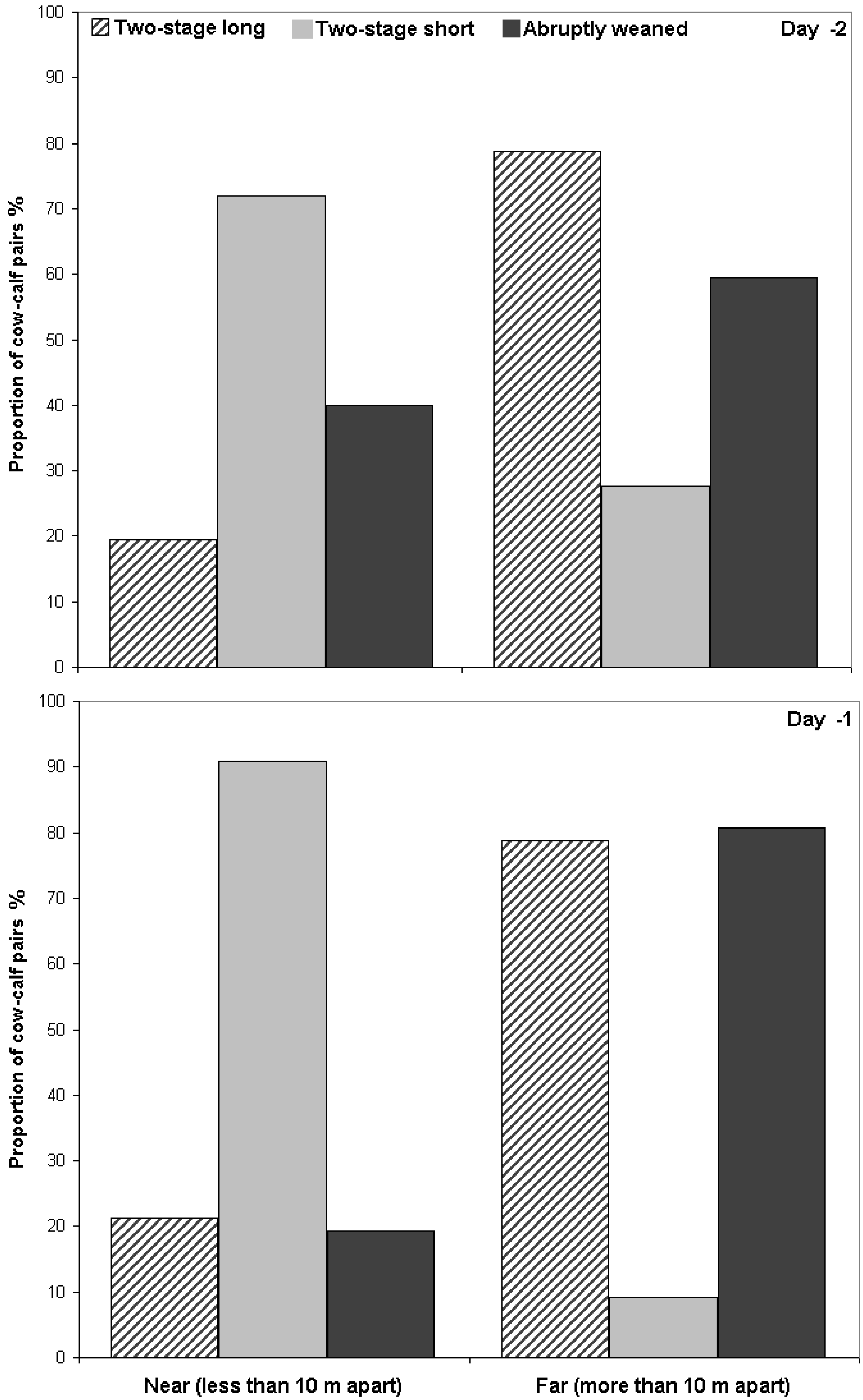
5.5.1 Trial 1

5.5.1.1 Behaviour

During the 2 d prior to separation, the proportion of calves less than 10 m from their dams and the proportion of calves greater than 10 m from their dams differed ($P<0.001$) among the three treatment groups. Results were similar on both days of observation (Figure 5.2). Calves from the short two-stage weaning treatment (calves most recently prevented from nursing) were found in closer proximity to their dams than calves from the other two treatment groups.

Observations of calf behaviour on d 2 and d 3 after separation revealed that control calves produced 41.9 calls/h, roughly 20 times more than the average of those calves weaned in two stages (1.4 calls/h, $P<0.001$; Figure 5.3). There were no treatment differences in calling behaviour ($P>0.48$) of calves separated after 14 d without nursing and those separated after 3 d without nursing. Call rates for both long and short two-stage groups were low (1.7 and 1.1 calls/h, respectively). During the 20 h observed,

Figure 5.2. Proportion of calves that were observed near their dam (≤ 10 m) and far from their dam (> 10 m) for cow-calf pairs in the long two-stage (nose-flaps on calves for 14 d before separation), short two-stage (nose-flaps on calves for 3 d before separation), and control (traditional weaning by separation) treatment groups. Data were recorded during morning observations on the 2 d prior to separating calves from their dams. On d -2, two days prior to the separation of cows and calves, observed values differed (P<0.001) from the values expected by chance (χ^2=18.6, 2 df, n=111). On d -1, one day prior to separation, observed values also differed (P<0.001) from the values expected by chance (χ^2 =44.0, 2 df, n=97).


Two-stage long
Two-stage short
Abruptly weaned
Day -2
Day -1
Proportion of cow-calf pairs %
Proportion of cow-calf pairs %
Near (less than 10 m apart)
Far (more than 10 m apart)
0
10
20
30
40
50
60
70
80
90
100

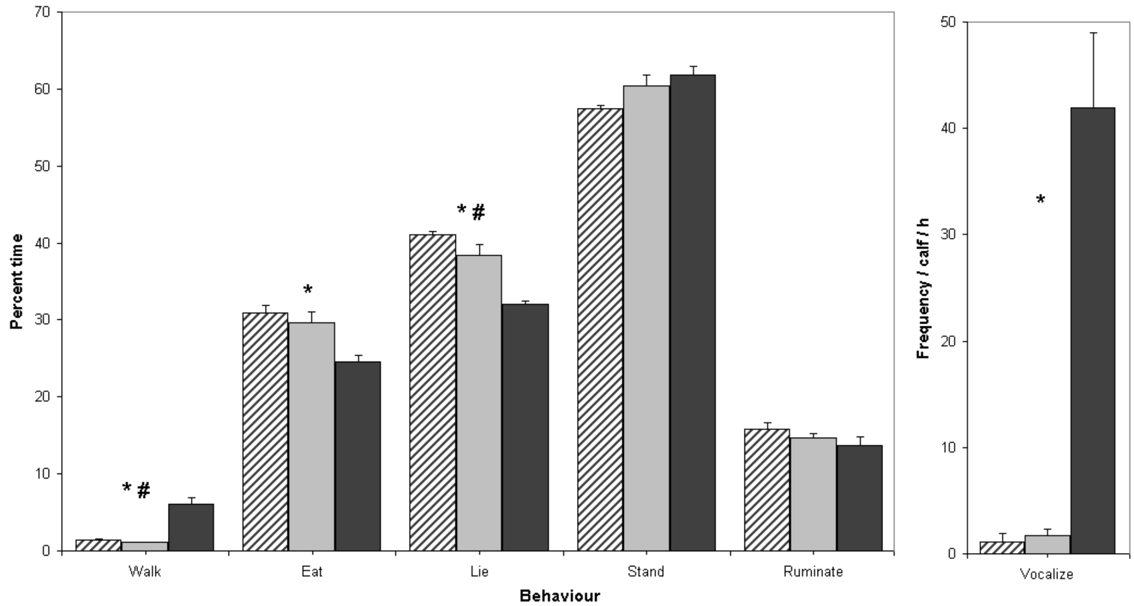


Figure 5.3. Impact of three weaning procedures on the percentage of time (mean ± SD) calves spent performing each behaviour during the second and third day after calf removal from their dams in Trial 1. Calves were either weaned in two stages or by the traditional method of separation (control). Calves in the two-stage weaning treatments were fitted with a nose-flap, anti sucking device that prevented nursing. Nose-flaps were applied for 14 d (▨ long two-stage; n=30) or 3 d (□ short two-stage; n=30) prior to the removal of calves from their dams. The control treatment (■ n=30) used the traditional approach of removing calves from their dam without prior prevention of nursing. Behaviour patterns were observed for a total of 20 h when calves were in dry-lot pens. Bars with a * differed (P<0.05) between two-stage weaning treatments (long and short two-stage treatments pooled) and controls. Bars with # differed (P<0.05) between the long and short two-stage weaning treatments.

calves weaned in two stages also spent less time walking (14 d two-stage=34.8 min, 3 d two-stage=26.9 min) compared to control calves (146.3 min, $P<0.001$; Figure 5.3). Two-stage calves spent more time lying down after separation ($P<0.001$). While control calves lay on average for 12.8 h of the 20 h observed, long and short two-stage calves lay for an additional 3.6 and 2.6 h, respectively. Two-stage calves also spent more time eating than the control calves ($P<0.001$). During the 20 h observed, control calves spent 9.8 h eating while calves from the long and short two-stage groups spent 12.4 and 11.8 h eating, respectively; roughly a 23% greater amount of time spent eating for calves weaned in two stages.

Regarding differences between the two-stage weaning treatments after separation, calves prevented from nursing for longer (14 d) spent more time walking (an additional 4.0 min over 20 h observation; $P<0.01$; Figure 5.3) and more time lying (an additional 31.8 min over 20 h of observation; $P<0.05$; Figure 5.3) than two-stage calves prevented from nursing for 3 d.

5.5.1.2 Growth rate

During the 14 d prior to separation, the ADG of nursing control calves was greater ($P<0.001$) than calves in either of the groups prevented from nursing for some portion of that time (Figure 5.4). Calves prevented from nursing for 3 d had a greater ADG ($P<0.001$) than those calves prevented from nursing for the full 14 d period (d -14 to d 0).

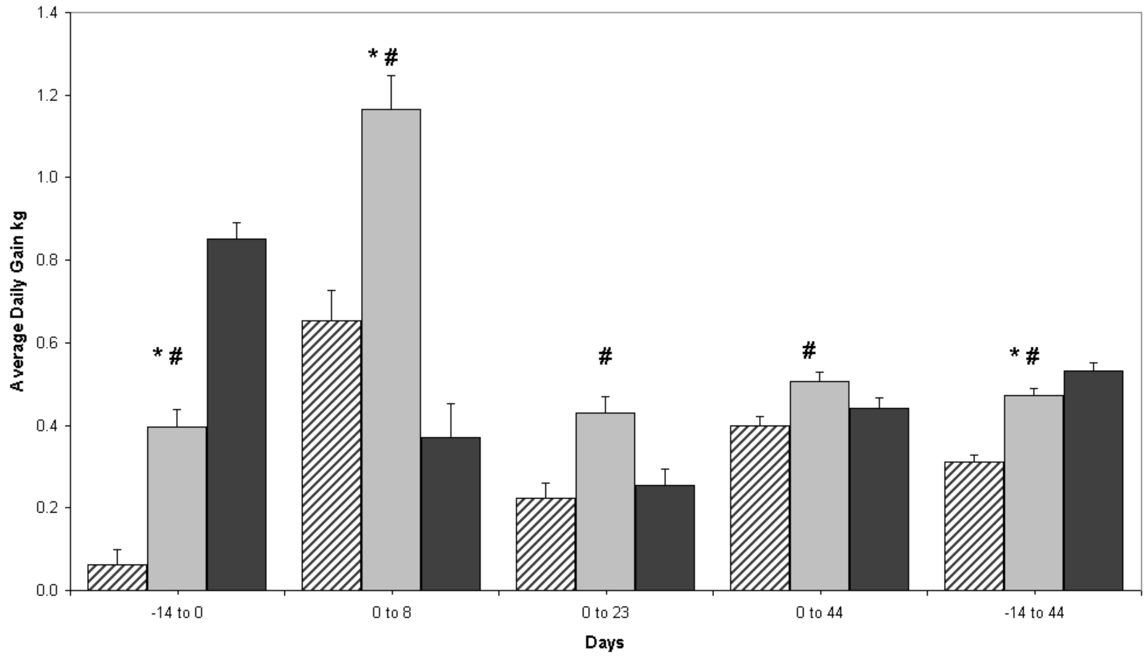


Figure 5.4. Least-square (LS) means (± SE) of ADG for calves in Trial 1 that were weaned in two stages or weaned by the traditional method of separation (control). Two-stage treatment calves were prevented from nursing by placing a nose-flap, anti sucking device, for 14 d (▨ long two-stage, n=57), or 3 d (□ short two-stage, n=58). The control treatment (■ n=73) used the traditional approach of removing calves from their dam without prior prevention of nursing. Data are presented for: 14 d prior to separation (d -14 to 0), the first 8 d following separation (d 0 to 8), 23 d following separation (d 0 to 23), 44 d following separation (d 0 to 44), and from 14 d prior to separation until 44 d after separation (d -14 to 44). Bars with a * differed ($P<0.05$) between controls and two-stage treatments (long and short two-stage treatments pooled). Bars with # differed ($P<0.05$) between the long and short two-stage treatments.

During the first 8 d following separation, however, calves from both two-stage treatment groups gained more weight (*P*<0.001) than control calves (Figure 5.4). Furthermore, short two-stage calves gained more weight than calves in the long two-stage group (*P*<0.001) during that first week after separation. From d 0 to d 44 calves weaned in two stages gained the same (*P* > 0.10) as the control calves. The long two-stage calves, however, gained less weight (*P*<0.01) than calves in the short two-stage group during this period. Over the whole trial (d –14 to d 44), control calves had a greater (*P*<0.001) ADG than calves weaned in two stages, and ADG of short two-stage weaned calves was greater (*P*<0.001) than the long two-stage treatment. In a comparison from the end of nursing to the end of the study period, ADG of long two-stage calves (0.31 ± 0.02 kg/d) was less (*P*<0.001) than the other two treatments, but there was no difference (*P*=0.09) in ADG between the short two-stage calves (0.39 ± 0.02 kg/d) and controls (0.43 ± 0.02 kg/d).

5.5.2 Trials 2 and 3

In Trial 2, during the period when two-stage calves were prevented from nursing, ADG of two-stage calves was similar to calves that were nursing (*P*=0.86; Table 5.1). In contrast, ADG of two-stage calves during the period when they were prevented from nursing in Trial 3, was lower than ADG of control calves (*P*=0.003). During the first week after separation, however, two-stage calves gained 0.42 kg/d more (*P*=0.001) than the control calves, when Trials 2 and 3 were combined (Table 5.1). When evaluated over the 28-d period after separation, ADG did not differ (*P*=0.67) between weaning

Table 5.1. Average daily gain (kg/d) of calves weaned by separation (control) or in two stages with nursing deprived for 5 d prior to separation (d 0) in Trials 2 and 3

Trial – Farm	Days[a]	Control	Two-stage	SE	*P*-value
Trial 2 – Termuende[b\]	-5 to 0	1.04	1.09	0.21	0.86
	0 to 7	0.66	0.91	0.11	0.10
n=100	0 to 28	0.99	0.94	0.07	0.67
	-5 to 28	0.99	0.96	0.06	0.70
Trial 3 – Goodale[c]	-5 to 0	1.52	0.59	0.21	0.003
	0 to 7	1.17	1.84	0.14	0.001
n=52	0 to 28	0.65	0.94	0.09	0.03
	-5 to 28	0.78	0.89	0.07	0.29
Trial 2 & 3 – Combined	-5 to 0	1.15	0.92	0.16	0.30
	0 to 7	0.95	1.37	0.09	0.001
n=152	0 to 28	0.85	0.92	0.06	0.40
	-5 to 28	0.90	0.92	0.04	0.73

[a] Average daily gain was measured for the 5 d before separation (-5 to 0), the 7 d following separation (0 to 7), for 28 d following separation (0 to 28) and from 5 d before separation to 28 d after separation (-5 to 28).

[b] Trial 2 was conducted with an equal number of steers and heifers at the Termuende farm.

[c] Trial 3 was conducted with heifer calves at the Goodale farm.

treatments in Trial 2, but in Trial 3, ADG was greater (*P*=0.03) for calves weaned in two stages compared to control calves. When the entire study period was considered (d –5 to d 28), ADG was not affected (*P* > 0.10) by the weaning treatments in either trial or when data from Trial 2 and 3 were pooled and analyzed together. If ADG is compared from the end of nursing until the end of the study, two-stage calves (0.88 ± 0.08 kg/d) had a greater ADG (*P*=0.03) than control calves (0.78 ± 0.08 kg/d) in Trial 3, but ADG was similar among treatments in Trial 2 (*P*=0.84).

5.5.3 Trial 4

There were no treatment differences (*P*=0.38) in the number of steps taken by calves when all pairs were nursing (Figure 5.5). During the 4-d period when two-stage calves were prevented from nursing they took more steps than their nursing counterparts (*P*<0.05), on average 2019 more steps/d. Applying a standard calf stride length of 65 cm, this is equivalent to 1.3 km/d. On the first 4 d after separation, control calves took an average of 8887 steps/d more than two-stage calves (*P*<0.05), which is equivalent to 5.8 km/d if the same stride length is applied. On the day following separation (d 1), control calves walked approximately 11.5 km/d (17637 steps/d) more than calves weaned in two stages (Figure 5.5). The magnitude of treatment differences in the distance traveled decreased (*P*<0.06) after 48 following separation (d 2). Over the period from 4 d before to 4 d after separation, two-stage calves took 4084 fewer steps/d, or walked an estimated 2.7 fewer km/d (*P*<0.01) than control calves.

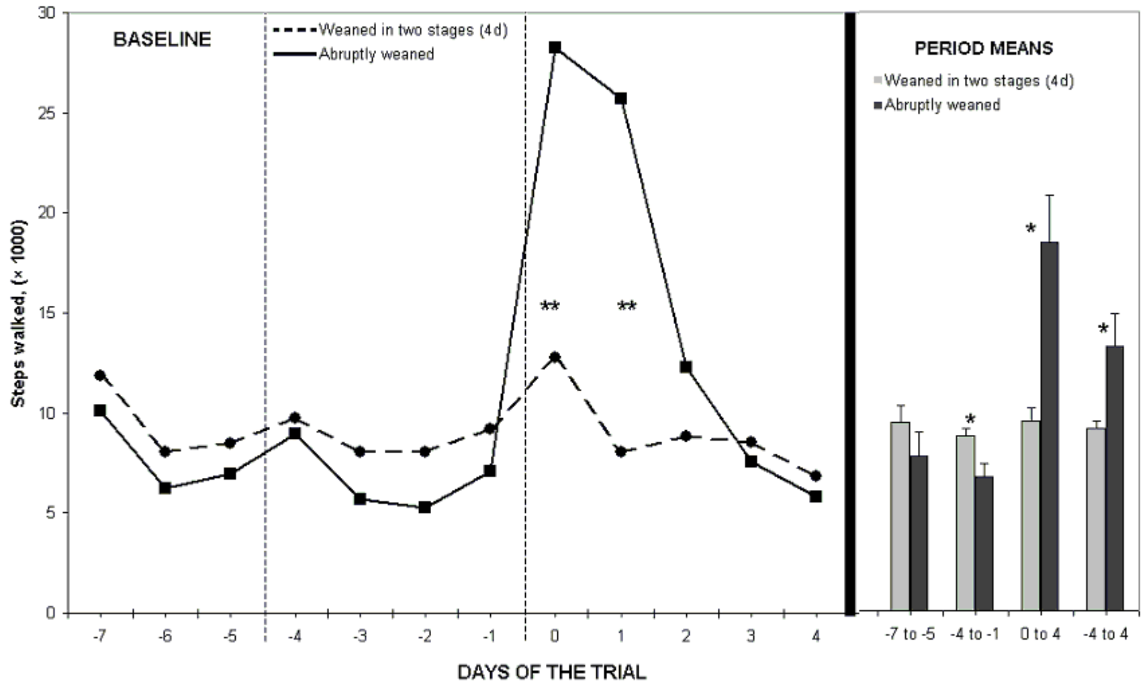


Figure 5.5. Mean (±SE) number of steps taken for each day of Trial 4 by calves weaned in two-stages and by the traditional method of separation (control). Two-stage treatment calves (n=5) were prevented from nursing by placing a nose-flap, anti sucking device for 5 d before separation and control calves (n=5) were weaned by the traditional approach of removing calves from their dam without prior prevention of nursing. Overall treatment effects are presented on the far right, for the baseline period when all pairs were free to nurse (d -7 to -5), the 4 d period when two-stage calves were prevented from nursing (d -4 to -1), the 5 d after all calves were separated from their dam (d 0 to 4), and the overall study (d -4 to 4). Differences between treatments on specific days are indicated by ** (*P*<0.001), and treatment differences within each of the four periods is indicated by * (*P*<0.05).

5.6 DISCUSSION

In Trial 1, I confirmed previously anecdotal observations (Chapter 3) that cow-calf pairs spend their time in closer physical proximity to one another when nursing is prevented than pairs whose calves can still nurse, at least during the short-term period after nursing is terminated (2 d in this study). In contrast, calves that had been prevented from nursing for a longer period (12 and 13 d) were observed to be at a similar distance from their dams as calves that could nurse, were from their dams. Previous research has reported that preventing nursing between pairs, but allowing them all other forms of social interaction, results in relatively subtle behaviour changes (Veissier and le Neindre, 1989; Chapter 3). The previous studies were all conducted under dry-lot pen conditions, and data presented here represent the first observations of pairs prevented from nursing, under more natural pasture conditions.

The distance between a cow and her calf has been reported to increase with time since their last nursing, up to a critical point, after which the individuals initiate reunion by increasing the time they spend walking and vocalizing (Watts, 2001). Nursing may decrease the motivation of cows and calves to stay close together. Physical proximity between dam and offspring has also been suggested as a possible measure of the attachment that exists between a cow and a calf (Gubernick, 1981). Maintaining a closer distance is assumed to reflect a stronger bond. Maintaining proximity in the present context might also reflect increased motivation to nurse. It is not clear whether the dam or the offspring may be more responsible for maintaining this close physical contact.

Observations following separation of the cow and calf in Trial 1 are similar to those reported in Chapter 3. Both studies clearly demonstrate that calves weaned in two stages vocalize less, walk less and spend more time eating and resting/lying after separation compared to control calves that are weaned by the traditional method of abrupt separation.

The process of transportation is assumed to be a significant compounding stressor that may contribute to the disruption of normal calf behaviour at weaning. However, in Trial 1, any effects of transportation on the behaviour of newly weaned calves did not negate the differences between control and two-stage weaning treatments in vocalization rate and time spent eating, resting and walking after separation. Similar to Trial 1, two-stage calves walked less than control calves for 2 d after separation in Trial 4.

Watts (2001), observing pairs that separated naturally under extensive pasture conditions, found both cows and calves increased their rate of vocalizing and spent more time walking, which culminated in reunion and nursing. Milk deprivation also results in increased vocalizing by young dairy calves, even when they are not being reared with their dam (Thomas et al., 2001). Certain behaviour patterns are mutually exclusive (e.g., walking and lying) and so not all changes in behaviour can be considered as independent. Reduced time spent eating and resting may be indirect results of calves spending more time vocalizing and walking.

The significant increase in walking behaviour by control calves in Trial 1 may be considered surprising given the limited space and stocking density (6 calves/5 × 10 m pen, or 8.3 m^2/calf). Regarding environmental effects on the response of calves to weaning, Price et al. (2003) found after traditional weaning by abrupt separation, that calves kept on pasture (6900 to 45700 m^2) walked significantly more than calves housed in dry-lot pens (288 m^2). Therefore, treatment differences in this trial may have been greater if calves were given more space to walk around.

Behaviour results from Trial 4 further emphasize the treatment effects on walking and the distance traveled by calves after separation. Walking behaviour quantified with pedometers designed for humans was in agreement with data collected previously by instantaneous scan sampling methods (Chapter 3). Unfortunately, pedometers cannot record the intensity of walking behaviour (e.g., whether calves moved at a trot or a slow walk) but they presented the opportunity to record walking 24 h/d, which is often logistically impractical by live observation. Although control calves walked less than two-stage calves during the period that nursing was prevented, the advantage of the two-stage treatment over the control after separation was much greater in magnitude. Over 4 d periods in Trial 4, the increase in walking by two-stage calves when nursing was deprived was less than one-third of the increase of walking by control calves after separation.

Results from the evaluation of ADG for calves were not consistent across all of the trials in this study, and there was limited evidence suggesting two-stage calves gain better compared to control calves, after separation. In all three trials, two-stage calves had improved performance during the first week after separation, and in Trial 3, two-stage weaned calves had greater ADG during the 4 wks after separation. Two-stage calves may have had greater ADG during the first week after separation because they spent more time eating than control calves, which were recorded vocalizing more frequently and spending more time walking during the second and third day following separation.

In two of three trials, control calves gained more weight than treated calves during the period when two-stage calves were being deprived of nursing. This is not surprising, as control calves would be expected to benefit from the nutrition in the milk they were receiving. In Trial 2, where ADG did not differ between weaning treatments, the quality of the pasture was better than in Trials 1 and 3. Availability of good quality pasture in Trial 2, may have allowed two-stage calves to immediately compensate for the loss of maternal milk. By comparison, poorer pasture conditions (dormant forage, moderate utilization levels) in Trials 1 and 3 may not have been sufficient to replace nutrients provided in the milk. It is proposed that low quality pasture was a major factor contributing to the large differences in ADG observed between the long two-stage weaning and control groups in Trial 1. These findings emphasize the fact that, at least nutritionally, two-stage calves should be considered weaned as soon as they are prevented from nursing. To ensure that ADG does not decline in Stage 1, nutritional management of two-stage calves should be carefully considered as soon as nursing is deprived, which was not done in any of the present trials.

Under the experimental designs reported here, control calves always had the advantage of a greater number of days nursing, which may also explain some of the inconsistencies in ADG among treatments. Perhaps another useful treatment group in the present trials could have been a second control group weaned on the same day that nursing was terminated for the two-stage calves, equalizing the number of days that calves spent nursing. Although the evaluation of ADG from the termination of nursing to the end of the study was potentially confounded by the number of days and management during the period before separation, only the long two-stage calves had lower ADG than calves weaned by traditional separation after nursing ended. In three separate trials, ADG for the two-stage calves (nose-flaps used for 3 to 5 d before separation) was equal or superior to controls when evaluated from the end of nursing.

Weaning by abrupt, remote separation typically imposes physical separation of the dam and offspring, which is very different from the natural weaning process. After prolonged physical separation, cows and calves invoke behavioural strategies such as increased vocalizing and increased walking, which help them reunite (Watts, 2001). Abrupt weaning by the separation of cows and calves activates these two primary behavioural response patterns. Two-stage weaning more closely simulates natural weaning by terminating nursing, albeit artificially, while the cow and calf are still together.

Despite some reservations from present trials regarding ADG when nursing is deprived, two-stage weaning represents a practical approach to minimize behavioural aspects of weaning distress in beef cattle. Nose-flaps are relatively inexpensive ($1.50 CAD) and can be reused after a recommended disinfection. Nose-flaps can be placed and removed in a few seconds if the calf is restrained (e.g., squeeze chute). The rate of retention for the nose-flaps in these studies was 95% or better.

Slight changes to the experimental design should be implemented in any further evaluations of ADG to equalize the number of days calves spend nursing. In addition, quality of the available nutrients should be carefully considered during the period when nursing has been deprived, and the time period that antisucking devices remain on calves prior to physical separation should be limited to 4 or 5 d. The possible implications of reducing weaning stress on the health of calves should also be further investigated. Although calves were the focus in the present series of trials, distance traveled and vocalizations by cows may also be reduced with two-stage weaning (Chapter 3), and the possible benefits for cows is worthy of investigation.

6.0 ALTERNATIVE METHODS FOR WEANING CATTLE: THE EFFECTS OF A TWO-STAGE PROCEDURE VERSUS PROVIDING FENCELINE CONTACT AFTER SEPARATION

6.1 ABSTRACT

The behavioural response of beef calves weaned in two stages was compared to the response of calves weaned abruptly, but given fenceline contact with their dams. Calves weaned in two stages (n=3 pens, each containing, 12 cow-calf pairs) were prevented from nursing for 4 d prior to separation from their dams. These calves wore a plastic antisucking device to prevent nursing. Calves weaned by fenceline contact (n=3 pens, each containing 10 cow-calf pairs) were allowed to nurse until they were separated into pens adjacent to their dams, separated by a wooden plank fence that permitted limited contact after separation but prohibited nursing. During the period when two-stage calves were prevented from nursing they were more likely to vocalize, less likely to be observed eating and more likely to be standing idle compared to controls still able to nurse. However, the actual numerical differences in behaviour were only slight relative to baseline activity levels. Treatment differences were more extreme on the 4 d that behaviour was observed following separation. Calves weaned in two stages vocalized 81.2% less ($P<0.05$). The rate of calling by fenceline calves across the 4 d following separation (14.9 calls/calf/hr) was more than 6 times that of two-stage calves. Two-stage calves spent 23.1% more time lying ($P<0.05$) and 60.3% less time standing ($P<0.05$) than calves weaned by fenceline contact. Providing fenceline contact between cows and

calves has been proven to reduce the behavioural signs of distress for calves compared with weaning by remotely separating pairs by a greater distance. However, weaning calves in two stages appears to still further reduce the distress of calves compared with fenceline contact weaning alone, and thus may further improve the welfare of calves at weaning.

6.2 INTRODUCTION

The standard method of weaning beef cattle is by abruptly separating cows and calves. Almost half of all the calves weaned in the United States are sold directly at weaning (USDA-NAHMS, 1998) and transported away from their home farm and thus are being weaned by remote separation from their dams. The abrupt and remote separation of cows and calves for weaning results in drastic changes to their normal behaviour patterns. Increases in the rate of vocalizing and time spent walking, and decreases in time spent eating and lying follow separation persist for at least three days following separation (Veissier et al., 1989; Stookey et al., 1997). As a consequence both the dam and offspring spend less time eating immediately after weaning is imposed (cattle: Chapter 3). This can result in reduced feed intake, reduced weight gain and even weight loss, in various species (cattle: Haley et al., 2005; horses: Houpt et al., 1984). Weight gain by beef calves after weaning is an issue of concern for production efficiency.

The extent of changes in behaviour caused by artificial weaning provides evidence that cows and calves are in a state of distress. Perhaps not surprisingly, the increased activity after weaning is associated with physiological changes that are also indicative of distress. Peripheral catecholamine concentrations in calves and epinephrine concentrations in their dams have been shown to increase following separation and subsequently show a significant decrease when the cows and calves are reunited (Lefcourt and Elsasser, 1995). Weaning has been shown to increase plasma or serum cortisol levels (McCall et al., 1987; Malinowski, 1990) as well as salivary cortisol levels in horses (Moons et al., 2005).

The stress of artificial weaning has been linked to immunosuppression (Griffin, 1989). Morbidity rates rise sharply among the offspring after artificial weaning (cattle: Harland et al., 1991). Fibrinous pneumonia ("shipping fever") mortality rates in calves are higher in feedlots containing a large proportion of recently weaned calves (Harland et al., 1991). The precise effects of weaning stress on morbidity after weaning may be confounded by mixing of unfamiliar animals (Ribble et al., 1995).

Efforts to reduce the stress of weaning have been tried with different farm animal species, leading to various alternatives to abrupt weaning by separation. In sheep, repeated separations of dams and their offspring for progressively longer periods have been tried in order to habituate them to being apart before their final separation for weaning (Orgeur et al., 1999). Artificial weaning often results in calves being segregated into homogenous groups, which causes significant disruption to their social organization (Veissier and le Neindre, 1989). Some alternative methods of weaning have focused on minimizing disruption to the social environment. Removing only a few dams at a time from the established social group has met with some success in terms of reducing the response to separation. This has been studied in elk (Church and Hudson, 1999), and horses (Holland et al., 1996). Another technique that has been tried as a way to stabilize social structure and help calves adapt to an unfamiliar environment after weaning, is the provision of resident trainer animals as pen mates for calves (Loerch and Fluharty, 2000; Gibb et al., 2000). However, this practice has not been shown to greatly alter the behaviour of calves. Changing the physical environment can have an impact on the behaviour of animals (sheep: Done-Currie et al., 1984). Therefore some cattle producers leave calves in the location familiar to them, and remove the cows when weaning.

One alternative to the traditional method of abruptly weaning by separation that has shown consistent beneficial effects, is to provide the dam and offspring limited physical contact at a fenceline after separation. One of the earliest available references investigating this method found fenceline weaned calves gained more weight initially but that there was no treatment difference compared to remote separation a few days after weaning (Nicol, 1977). Though the author noted that cows and calves separated into adjacent paddocks seemed less disturbed by the weaning process compared to remotely separated pairs, no behaviour observations were recorded. Studies since have shown that, compared with remote separation, allowing fenceline contact between dam and offspring reduces the behavioural response to weaning (horses: McCall et al., 1985; elk: Haigh et al., 1997; cattle: Stookey et al. 1997, Price et al., 2003). Specifically with cattle, calves have been shown to spend less time walking and more time resting, compared with remotely weaned cattle (Stookey et al., 1997).

Weaning cattle in two stages has also been shown to reduce the behavioural response of cows and calves to artificial weaning (Haley et al., 2005; Chapter 3). Preventing nursing between cow-calf pairs (Stage 1) prior to separation of the cow and calf (Stage 2) reduces the overall changes in behaviour compared with abrupt weaning. Nursing need only be prevented for a few days prior to separation to observe beneficial effects on behaviour (Haley et al., 2005).

The objective of the present experiment was to compare the behavioural responses of beef calves weaned in two stages with calves weaned abruptly, by separation that permitted them fenceline contact with their dams.

6.3 MATERIALS AND METHODS

All experimental procedures used in this study were approved by the University of Saskatchewan's Committee on Animal Care and Supply (UCACS Protocol #20000096) and animals were cared for according to Guidelines set by the Canadian Council on Animal Care (1993).

6.3.1 Animals

A total of 66 cow-calf pairs were moved from grazing pastures into dry-lot pens (30.5 × 27.5 m) 7 d prior to the start of the experiment. Pairs had access to water *ad libitum* and were fed free-choice grass hay, which was replenished to individual pens, as necessary.

6.3.2 Treatments and experimental design

Pairs were randomly assigned to one of two treatments and weaned either in two stages with nursing deprived for 4 d prior to separation (n=3 pens, 12 pairs/pen), or weaned abruptly with fenceline contact (n=3 pens, 10 pairs/pen). Both treatments and pens were balanced for sex of the calves. During preliminary testing some calves managed to nurse while wearing the antisucking device so each pen of two-stage animals contained two additional cow-calf pairs to help ensure adequate numbers of pairs successfully received the intended two-stage treatment.

Four days prior to separation, all calves were run through a handling facility and calves on the two-stage weaning treatment were fitted with a lightweight plastic antisucking device (Quietwean nose flap, JDA Livestock Innovations, Saskatoon, Canada), which prevented nursing (Figure 6.1). The device was similar in shape and size to those used in previous experiments on two-stage weaning (Chapter 4, 5) except it did not have barbs along on the front plate or around the outside edge. The device was also made of flexible plastic that was twisted to expand the gap opening, which allowed it to be placed in the calf's nose where it hung freely without piercing the nasal septum. The device prevented nursing by covering the calf's mouth as the neck was extended and the head lowered toward the udder thus obstructing access to the dam's teats. Calves were able to consume hay and drink water while wearing the device. Calves from the fenceline contact treatment were restrained and manipulated for a comparable amount of time but were not fitted with an antisucking device.

Figure 6.1. Photo of the antisucking device (Quietwean nose flap, JDA Livestock Innovations, Saskatoon, Canada) worn by calves to prevent nursing.

On the day of separation, all calves were again moved through the handling facility and the antisucking devices were removed from calves in the two-stage group. Calves were returned to their home pens, however in their absence, dams of two-stage calves had been removed and walked to pasture, while dams from the fenceline treatment group were moved into pens adjacent to their respective home pen. Pairs from both treatment groups were within auditory range of one another, but fenceline pairs additionally had visual and limited physical contact through a wooden plank fence (Figure 6.2).

6.3.3 Behaviour observations

Behaviour was observed for 9 d in this study. To begin, all cows and calves were observed for one day to obtain baseline information about their activity levels and to be sure that all pairs were still nursing (Period 1). Observations over the next 4 d documented the response of the two-stage pairs when nursing was prevented (Period 2). Lastly, cows and calves from both treatments were separated and their behaviour was observed for another 4 d (Period 3, see Figure 6.3).

The same observational methods were used on every day. Each pen of animals was observed from 14:30-18:30 hours (4 h) by instantaneous scan sampling, every 10 min. One observer was able to record the number of calves walking, lying, standing, drinking water, eating, nursing (calf having a teat in it's mouth), and ruminating. Calves observed standing or lying could also be recorded as ruminating. During the period that two-stage calves were wearing antisucking devices, instantaneous scan sampling was also used to

Figure 6.2. Photo showing contact between a cow and calf in the fenceline contact weaning treatment through the wooden plank fence.

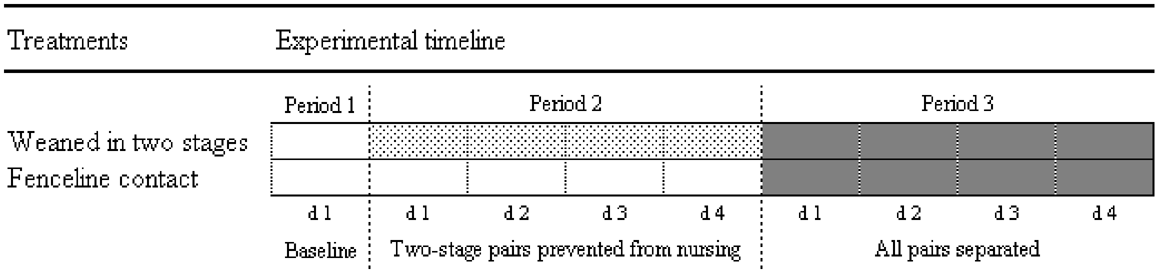


Figure 6.3. Treatments and the experimental timeline for the present study, illustrating the days when cows and calves were able to nurse □, days when two-stage cows and calves were together, but prevented from nursing ▩, and days when the cows and calves were split apart ■. During the period of separation, two-stage calves were far from their dams, while fenceline contact calves were in pens adjacent to their dams. Pairs from both treatment groups were within auditory range, while fenceline pairs additionally had visual and limited physical contact through a wooden plank fence.

to record their attempts to nurse when the calf had its head or nose in contact with the cow's udder at the first instant of the sampling interval.

For 2 min during each 10-min interval the total number of vocalizations by calves in each pen was recorded. Any audible vocal sound that could be attributed to a calf was counted as a vocalization. Bursts of vocalizing were recorded by counting the number of individual short successive calls within each sequence, as distinguished by inhalations taken by the animal between each separate call (see Kiley, 1972, See-saw calls - type B, p. 193).

6. 3.4 Statistical analysis

Pens were the experimental unit in this study. Daily frequency counts for each behaviour variable and the total numbers of calf observations were summed for each pen, for use in the statistical analyses. All data were analyzed using a generalized estimating equations (GEE) method to account for repeated measures within pen. Data were analyzed using a statistical computer software program (SAS v.8.2 for Windows (PROC GENMOD); SAS Institute, Cary, North Carolina, USA). Model specifications included a binomial distribution, logit link function, Repeated statement with subject equal to pen number, and an AR(1) correlation structure. Variables remaining in the final multivariable model at $P<0.05$, based on the robust empirical standard errors produced by the GEE analysis, were considered statistically significant. Since vocalizations were recorded as a continuous variable they were analyzed with a Poisson distribution and log link function.

Treatment effects and day effects were analyzed within specific time periods: 1) the baseline period when all pairs were nursing, 2) the period when two-stage pairs were prevented from nursing, and 3) the period after cows and calves were separated. To gauge the collective response of cows and calves to weaning, an overall treatment comparison was performed combining periods 2 and 3.

To help visualize the data, daily frequency counts and the total numbers of calves observed were used to calculate the rate of vocalizing (calls/calf/h), and the percent of observations that calves were observed performing each behaviour being recorded during the 4-h (240-min) daily observation period.

Data from 9 of the 36 calves assigned to two-stage weaning were discarded from the analyses for not having received the full-intended treatment. The present study was designed to investigate the effects of two specific weaning treatments, not the efficacy of the antisucking device being used. Accordingly, two-stage calves observed nursing while wearing the antisucking device, or calves that lost their device outside designated observation periods (and therefore may have nursed), were eliminated from the study. However, these calves and their dams were not physically removed from their established groups, which remained intact throughout.

6.4 RESULTS

All treatment effects and the complete behaviour results of calves within the 3 specific time periods considered are presented in Table 6.1.

6.4.1 Period 1 (baseline)

During the baseline period, there were no differences in behaviour between the two groups of calves.

6.4.2 Period 2 (Stage 1)

Calves prevented from nursing vocalized more frequently than calves that were still able to suckle ($P<0.001$), though their rates of vocalizing were similar to the levels recorded during baseline observations (two-stage baseline vs. Stage 1=0.9 vs. 2.1 calls/calf/h).

Across the four days when nursing was prevented two-stage calves were observed eating during 42.9% of observations, compared with 50.4% of observations for calves that were still nursing ($P<0.001$). Two-stage calves spent 27.9% of their time lying while they were being prevented from nursing while the control calves lay for 24.9% of the observed time ($P<0.001$). Control calves spent 3.9% of the observed time nursing while the two-stage calves spent a smaller proportion of their time (2.2%) engaged in nursing attempts (head in contact with the udder, $P<0.001$).

Table 6.1. Mean (± SE) percentage of the total observed time in which calves from two-stage and fenceline weaning treatments were exhibiting behaviour variables of interest. The rate of vocalizing is expressed in calls/calf/h. Analyses were performed within specific time periods: 1) baseline period when all pairs were nursing, 2) the period when two-stage pairs were prevented from nursing, 3) the period after cows and calves from both groups had been separated. Treatment means separated by * differ by $P<0.05$; ** by $P<0.01$; *** by $P<0.001$.

Period Behaviour	Two stage weaning 4 d	*P* -value	Fenceline contact
Period 1 (baseline)			
Vocalizing	0.9 ± 0.3		1.5 ± 0.7
Walking	3.1 ± 0.0		4.3 ± 0.9
Lying	23.1 ± 1.7		22.4 ± 4.6
Standing	21.8 ± 0.9		20.1 ± 2.9
Drinking	0.6 ± 0.2		0.8 ± 0.2
Eating	47.8 ± 2.8		49.2 ± 3.7
Nursing	3.5 ± 0.3		3.2 ± 0.3
Ruminating	10.1 ± 0.5		7.7 ± 1.4
Period 2 (two stage pairs prevented from nursing)			
Vocalizing	2.1 ± 0.4	***	0.4 ± 0.1
Walking	2.1 ± 0.3		2.4 ± 0.5
Lying	27.9 ± 2.4	*	24.9 ± 1.7
Standing	24.2 ± 2.2		18.2 ± 1.9
Drinking	0.8 ± 0.2	*	0.3 ± 0.1
Eating	42.9 ± 2.4	***	50.4 ± 1.2
Nursing	2.2 ± 0.4	***	3.9 ± 0.3
Ruminating	13.2 ± 1.8		11.4 ± 1.0
Period 3 (all pairs separated)			
Vocalizing	2.8 ± 0.4	***	14.9 ± 4.1
Walking	1.5 ± 0.3		1.9 ± 0.4
Lying	44.7 ± 2.6	***	36.3 ± 2.8
Standing	13.1 ± 0.6	***	21.0 ± 2.6
Drinking	0.6 ± 0.2		0.8 ± 0.1
Eating	40.1 ± 2.4		39.9 ± 1.8
Nursing	na ± na		na ± na
Ruminating	19.9 ± 1.4		20.2 ± 1.9

6.4.3 Period 3 (Stage 2: following separation)

On the four days behaviour was observed following the separation of cows and calves, two-stage calves called less than those calves weaned with fenceline contact (2.8 vs. 14.9 calls/calf/h, $P<0.001$). Calling by both two-stage and fenceline treatment groups was highest on the first day of separation (8.1 vs. 30.1 calls/calf/hr, respectively). Thereafter calling declined, however, four days after the separation of cows and calves treatment differences in the rate of vocalizing could still be detected (0.1 vs. 1.6 calls/calf/h, two-stage vs. fenceline, respectively; Figure 6.4). Two-stage calves were observed lying more often following separation than fenceline calves (44.7 vs. 36.3% of observations, $P<0.001$), and as with vocalizing, this effect was strongest on the first day of separation (89.9 vs. 51.2 min; Figure 6.5). This resulted in a significant difference in the percentage of time calves spent standing, with fenceline contact calves spending more time standing than two-stage calves 21.0 vs. 13.9 % of observations ($P<0.001$).

Overall response to weaning

Combining Stages 1 and 2, two-stage calves called less ($P<0.001$) and spent more time lying ($P<0.001$) than calves weaned with fenceline contact.

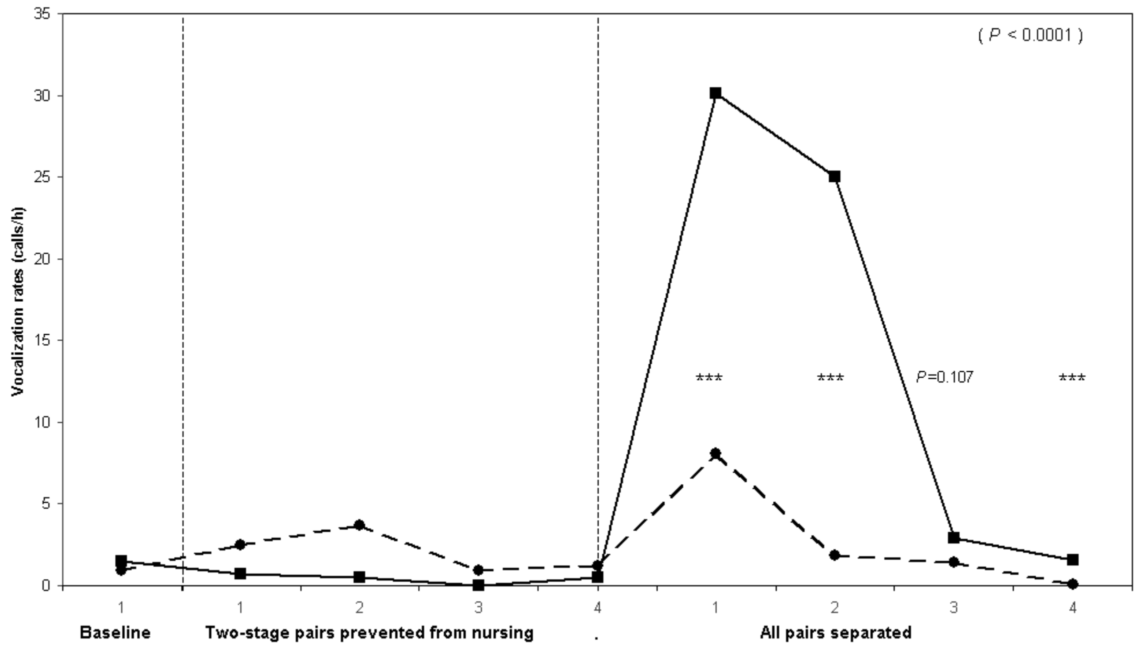


Figure 6.4. Mean vocalization rates[1] for calves (calls/calf/h) on each day of the experiment. Calves were either weaned in two-stages (– – ● – –) (deprived nursing for 4 d prior to separation), or weaned with fenceline contact (—■—). Italicized *P*-values in parentheses indicate significant treatment effects within each period. Treatment effects on specific days of the experiment are shown by asterisks: *P*<0.001 (***). Statistical tendencies on specific days are indicated by the actual *P*-value. [1]Call rates were calculated based on the number of calls recorded during a 2-min sampling period, taken every taken every 10 min.

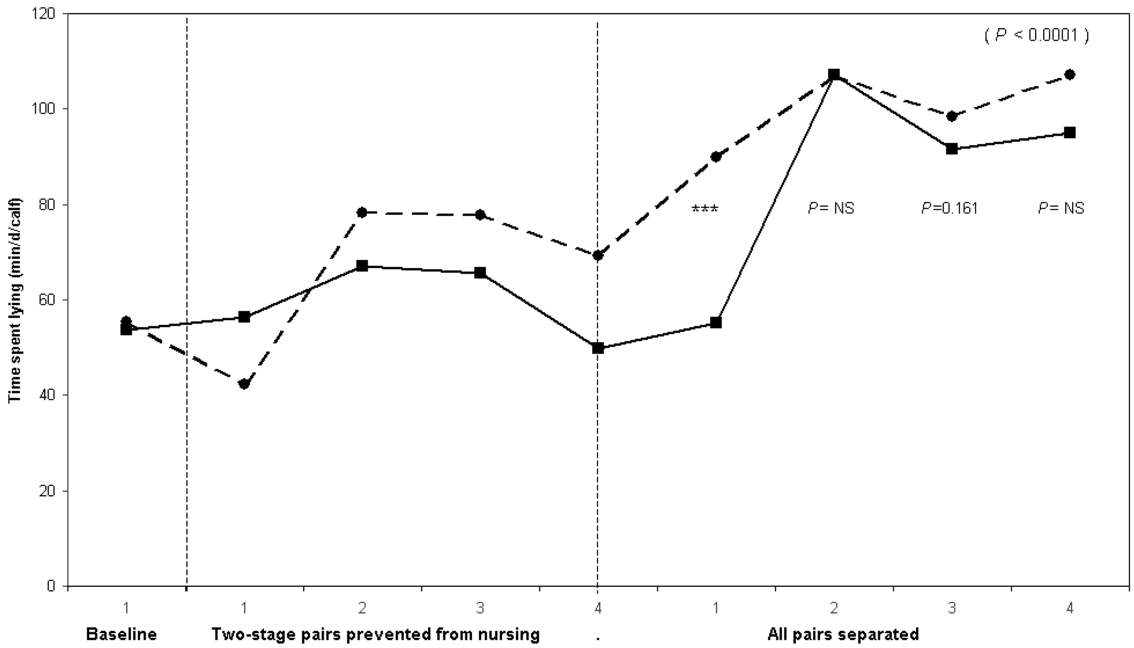


Figure 6.5. Mean time[1] (min) calves spent lying during 4 h of observation from each day of the experiment. Calves were either weaned in two stages (– – ● – –) (deprived of nursing for 4 d prior to separation), or weaned with fenceline contact (——■——). Italicized *P*-values in parentheses indicate significant treatment effects within each period. Treatment effects on specific days of the experiment are shown by asterisks: *P*<0.001 (***). Statistical tendencies on specific days are indicated by actual *P*-values.

[1]Time was calculated based on scan samples recorded at 10-min intervals.

6.5 DISCUSSION

The results point to noticeable and significant differences between the behavioural response of calves to weaning in two stages and weaning by fenceline contact. Calves weaned in two stages called less, spent more time lying and less time standing compared to calves that were abruptly weaned, but allowed fenceline contact with their dams following separation. Previous research has shown that fenceline contact improves the response of calves compared with abrupt weaning followed by moving cows and calves far away from each other (Stookey et al., 1997; Price et al., 2003), but the present results suggest that weaning calves in two stages reduces behavioural signs of distress even further.

During the period when they were prevented from nursing, two-stage calves were more vocal than calves that were still able to nurse, but calling was still quite infrequent (2.1 calls/calf/h). This result is in agreement with previous work, which reported similarly low rates of vocalizing by calves when they were prevented from nursing (e.g., 2.6 calls/calf/hr, Chapter 3). During the period that two-stage calves were prevented from nursing they spent about 15% less time eating than controls. In other studies, calves prevented from nursing spent the same amount of time eating as calves that were still able to nurse (Chapter 3). One difference here was that calves were being kept at a higher stocking rate than previous studies (22 vs. 16 animals/pen). This may have increased competition for space available at the round-bale hay feeder. As the nasal septum is quite a sensitive area two-stage calves may have been deterred from competing for space if they were bumped on the nose. Future observations of calf behaviour in and around the hay feeder could help to further clarify whether this is the case as well as to determine whether the antisucking devices affect feed intake.

Differences between the treatments were most striking after the cows and calves were separated. Two-stage calves called less and spent more time lying while calves weaned with fenceline contact spent more time standing. The rate of vocalizing by two-stage calves (2.4 calls/calf/hr) was similar to their calling rate in response to the termination of nursing. The rate of calling by fenceline calves across the 4 d following separation (14.9 calls/calf/hr) was more than 6 times that of two-stage calves. Controlled studies show calves weaned by fenceline contact call less, walk less and spend more time lying than calves remotely separated from their dams (Stookey et al., 1997; Price et al., 2003). Time spent walking in this study was similar for two-stage and fenceline contact weaning groups.

Both vocalizing and walking appear to be functional responses to separation for the cow and calf, to help them reunite. Watts (2001) observing free ranging cows and calves that became visually separated from one another naturally while grazing, found that time spent walking and the rate of vocalizing were inversely related to the amount of time a cow and calf pair took to reunite. Reduced time spent eating and lying might reflect limitations of the behavioural system in terms of their basic time budget.

Observations of weaning calves in two stages strongly suggest that the rate of vocalizing following abrupt weaning is a response caused by the synergistic effects of perhaps two primary motivations: 1) to locate the dam and 2) to nurse. If so, this could help to explain why calves weaned with fenceline contact call less than remotely weaned calves as they have already located their dams. In this respect the situation of fenceline contact can be considered similar to the initial phase of two-stage weaning; in both cases nursing is prevented while the dam is still present. However, the behavioural responses of calves to these two situations suggest they are not equal. With two-stage weaning cow-calf pairs are able to maintain and engage in extensive physical contact when nursing is prevented whereas fenceline weaning offers less contact when nursing is prevented. If physical contact is an important psychological necessity for the pairs then two-stage weaning has a clear advantage.

A fenceline may represent a psychological barrier for cows and calves. It could be interesting to explore the possibility that varying degrees of visual obstruction, for example with more or less porosity, may affect the animals' perception of being near to their partner. Varying the degree of visual obstruction might alter the response of cows and calves to separation by fenceline contact. A limited length of fenceline between adjacent pens could reduce the space available for cows and calves to see each other or make contact at the fence without being obstructed by other animals. Though it did prevent nursing, the wooden plank fences used in the present study did permit some contact between dams and their calves in the adjacent pen, such as grooming, through spaces in the fence (see photo, Figure 6.2). These dam-offspring interactions were not recorded in this study but, in future, hypotheses could be tested about the degree or nature of fenceline contact and the individual variation in response to weaning by this method.

The particular factors that afford fenceline weaning its benefit over remote separation are unclear although different barriers from the ones in this study have also been successful at improving the response to weaning over remote separation. For example Price et al. (2003) used electrified wire, which provides almost no visual obstruction but may prevent any physical contact at the fenceline. This line of investigation could be pursued using partial contact at weaning to evaluate the importance of various components of dam-offspring interaction in the maintenance and dissolution of their relationship. For example, olfactory cues may play an important role in the benefits gained by providing fenceline contact. Research with pigs has documented beneficial effects of spraying synthetic maternal pheromones on the behaviour of newly weaned pigs (McGlone and Anderson, 2002).

Perhaps the most obvious difference between denying nursing by fenceline contact or an antisucking device is that the latter allowed more physical contact and interaction between the cows and calves. Specifically, fenceline calves are unable to engage in nursing attempts, which may potentially reduce or satisfy their motivation to nurse. This opportunity may be the critical feature primarily responsible for affording the benefits of two-stage weaning at reducing behavioural signs of distress compared to other weaning methods.

The reason for the slightly increased rate of vocalizing by two-stage calves when they were prevented from nursing is worth considering. Calves may vocalize to find their dam within the pen, so that they can attempt to nurse, as is the case under pasture conditions (Watts, 2001). However, despite the fact the spatial proximity of pairs was not recorded in this study it was noted that vocalizing also occurred while pairs were right next to each other. Vocalizations by calves might also be given as an indication of their biological need for the dam's milk (Watts and Stookey, 2000). Piglets isolated from the sow call more if they have gone unfed suggesting that their vocalizations provide reliable information about their need for the sow's resources (Weary and Fraser, 1995; Weary et al., 1997). If this is also true for calves it might be hypothesized that the calling rates of calves would vary depending on their level of nutritional dependence or the amount of milk they were receiving when nursing was prevented. All calves in the present study were 210 days of age or older and so no longer entirely dependent on their dam for their nutrition. This might in part explain the limited response when two-stage calves were prevented from nursing although no investigations have explored how varying milk quantity affects the response of calves to weaning.

The lower rate of vocalizing by two-stage calves following separation may reflect a lower motivation to locate their dams, reduced by the fact they had not nursed for 4 days. However, in previous studies calves deprived of nursing for 14 d still responded to being separated from their dams and their rate of vocalizing was similar to calves separated after 3 d without nursing (Haley et al., 2005). Thus the vocal response of two-stage calves to separation might also reflect a milder response to separation than when this is used concurrently to terminate nursing. Some calling may be the result of disruption to their established social environment or due in part to stimulation caused by the increase in vocalizing and general activity of the abruptly weaned fenceline animals in nearby pens.

After separation, calves weaned by fenceline contact also spent more time standing. It appeared this time spent standing was often close to the fence and may have represented time the calves spent trying to get close to their dam to try and suckle.

No difference was found between the time fenceline and two-stage calves spent eating after separation. Price et al. (2003) found that, compared with the remotely separated pairs, calves weaned with fenceline contact spent more time eating and that they had improved weight gain during the 52 weeks after pairs were separated. Given the present results regarding time spent eating I would expect calves from two-stage and fenceline weaning groups to be similar in the amount of weight gained although this was not studied in this experiment. However, behaviour differences aside from eating time might impact daily weight gain such as time spent resting and the rate of vocalizing.
Regarding the practical comparison of these two weaning methods it is apparent that weaning in two stages requires calves be handled once to fit an antisucking device and another time to remove it. The amount of stress cattle experience during handling will depend on many factors, for example the ability and attitude of the stockperson and the of aversion of animals to the techniques used (Rushen et al., 1999). Whether two-stage weaning results in additional handling may depend on specific farm practices. Most

management systems will handle calves at weaning for the purpose of identification and in some cases calves may also be handled and vaccinated up to two weeks prior to weaning. Furthermore, any potential stress associated with extra handling must be weighed against the stress that is caused by abrupt weaning. The effect of abrupt weaning on the behaviour of cattle is well documented and though less may be known about behaviour changes that result from handling there is no evidence of prolonged effects such as those caused by abrupt weaning.

Handling facilities are important for promoting safety for the animals and the handlers if two-stage weaning is incorporated. A lack of handling facilities may limit the feasibility of adopting two-stage weaning for some producers who either do not have such facilities or who graze their cattle on extensive rangeland pastures, far from any handling facilities. Although fenceline weaning does not necessarily require handling facilities it does require strong, secure fences as it is not uncommon for cows or calves or both, to break through barriers in their attempts to reunite, after being separated. It seems likely that weaning in two stages may help to reduce these sorts of destructive behaviour patterns. Further to the topic of input costs, weaning in two stages would require producers to invest in some kind of device to prevent nursing. However, in many cases these devices can be used more than one time.

Studies have shown that weaning cattle with fenceline contact appears to be an improvement over remotely separating cows and calves (Stookey et al., 1997; Price et al., 2003). The present study provides the first evidence that two-stage weaning offers a further improvement for the welfare of calves and the response of cows to these two situations must also be considered.

7.0 THE RESPONSE OF 5-WEEK-OLD NURSING DAIRY CALVES AND THEIR DAMS TO BEING WEANED IN TWO STAGES OR ABRUPTLY BY SEPARATION

7.1 ABSTRACT

This study examined whether a two-stage method of weaning would result in fewer behavioural signs of distress than abruptly weaning by separation for dairy cows and calves after nursing *ad libitum* from birth to 5 weeks of age. Sixteen Holstein dairy cow-calf pairs were used in this study. Half the calves (n=8) were weaned in two stages and so wore an antisucking device, which prevented nursing for the 4 d (Stage 1) just prior to physical separation (Stage 2). The other half of the cow-calf pairs were controls (n=8) and were abruptly weaned, being prevented from nursing by being physically separated. The behaviour of cows and calves from the two treatment groups was similar during baseline observations, when all pairs were able to nurse. The first day nursing was prevented two-stage cows behaved the same as they had during the baseline period. By the fourth day without nursing, two-stage cows had decreased their lying time by 8.3% (P<0.05). By comparison, calves responded immediately when nursing was prevented, spending more time attempting to nurse than the amount of time they spent nursing during baseline observations (P<0.05). Calves also increased their rate of vocalizing (P=0.08) over baseline. The rate of vocalizing by calves was similar on the first and fourth day without nursing (d 1=6.7 calls/h vs. d 4=4.1 calls/h, NS). By the fourth day without nursing, calves showed an increase in time spent eating (P<0.05) and

ruminating, over their first day without nursing (*P*<0.05). After separation, the rate of vocalizing tended to be higher for abruptly weaned cows (147.1 calls/h) than for cows weaned in two stages (74.6, *P*=0.08). The effect of treatment was of greater significance for calves, with two-stage calves vocalizing less than controls (2.2 vs. 51.8 calls/h, *P*<0.05) on the first day of separation. The two-stage cows and calves both spent more time ruminating after separation compared to abruptly weaned animals (cows=*P*<0.05; calves=*P*<0.001). Two-stage calves spent more time lying (*P*<0.05) while abruptly weaned calves appeared more agitated, spending more time moving (*P*<0.001) and making more attempts to jump out of the pens they were being kept in after separation. Two-stage calves were not observed trying to jump out of their pens (*P*<0.001). These results are similar to those found when weaning much older beef calves. Five-week-old dairy calves showed fewer behavioural signs of distress when weaned in two stages compared to those weaned abruptly, by separation despite being highly dependent on their dam's milk for their nutritional requirements.

7.2 INTRODUCTION

Most cattle are abruptly weaned by separating cows and calves. Their behavioural response to this event is highly predictable and remains noticeably different from baseline levels for 3 or 4 days (Vessier et al., 1989; Chapter 3). Compared to their activity levels while nursing, after abrupt weaning, cows and calves increase their rate of vocalizing, spend more time walking and less time eating and resting (Vessier and le Neindre, 1989; Stookey et al., 1997; Chapter 3). Disconnecting the two principle features of abrupt weaning by terminating nursing between pairs for a period (Stage 1) prior to physically separating the cows and calves (Stage 2), greatly reduces the behaviour signs of distress cattle normally display in response to weaning (Chapter 3). Weaning cattle in two stages is thought to mimic certain critical aspects of the natural weaning process, which serve to reduce behavioural signs of distress compared to weaning abruptly, by separation. A feature presumed to be important for the success of two-stage weaning is that nursing is terminated while the dams and calves are still together, allowing them an opportunity to engage in nursing attempts as well as other forms of social interaction.

If two-stage weaning suitably mimics some critical features of the natural weaning process then the underlying principles should apply to calves of different ages representing varying degrees of nutritional dependence on their dams. The reason for this is that the timing of weaning is not considered to be a constant or life history trait and rather is suggested to reflect more about local ecology (Lee, 1997). That is to say, the timing of weaning is not strictly determined by the age of the offspring per se but rather may also be driven by other factors such as the amount of nutrients available to the dam to support lactation. The progression and exact point of natural weaning is reported to vary even within species (Reinhardt and Reinhardt, 1981; Gonyou and Stookey, 1987). Thus there should be the plasticity to withstand weaning at various points in time.

Previous investigations of two-stage weaning have used calves ranging in age from 185 to 225 d of age (Chapter 3). At that point in time the dam's milk production is likely well into decline and artificial weaning is likely being superimposed over an underlying natural process. The objective of this present study was to examine whether the two-stage method would have similar beneficial effects on the response of very young calves to weaning as has been shown for older calves.

7.3 MATERIALS AND METHODS

All experimental procedures used in this study were approved by the University of British Columbia Committee on Animal and the University of Saskatchewan's Committee on Animal Care and Supply (UCACS Protocol #20000096) and animals were cared for according to Guidelines set by the Canadian Council on Animal Care (1993).

7.3.1 Animals and housing

This study was conducted between May and July 2002 at the University of British Columbia Dairy Education and Research Centre, Agassiz, Canada. Observations were conducted on 16 Holstein dairy cows (lactation range=1 to 6) and their calves, which were suckled and remained with their dam until separation at 31.5 days of age (range=27.3 to 37.1 days). No cows had any previous maternal experience beyond 24 h post-partum.

Parturition took place in individual maternity pens (4.3 × 3.0 m) where pairs remained until the calf was observed to be sucking successfully (range=24 to 72 h). Calves were given two supplemental 2-litre feedings of their dam's colostrum within the first 24-h period.

From the maternity pen, pairs were moved to free-stall housing (190 m^2) where all pairs in the experiment were kept together. Stalls were bedded with wood shavings and water and a total mixed ration (grain, maize silage and hay silage) were available *ad libitum*. The free-stall barn included a separate bedded area that was only accessible to calves, where they were provided *ad libitum* access to water, total mixed ration, tall fescue hay and calf starter ration.

Cows were milked twice daily throughout the experiment. This involved moving cows from the home pen to the milking parlour at 0700 and 1500 h. Cows were separated from their calves for approximately 1 hour at each milking.

7.3.2 Weaning treatments

Calves (7 females, 9 males) were assigned to one of two treatment groups, to be weaned either in two stages or weaned abruptly by separation. As much as possible groups were randomized to treatments while attempting to balance the sires and sex of the calves. Two-stage calves wore a lightweight plastic antisucking device to prevent nursing for 4 d (Stage 1) prior to the physical separation of cows and calves (Stage 2). The antisucking device (Figure 4.1; Villa Nueva S.A., Villa Maria-Cordoba, Argentina) was 12.0 × 7.5 cm at its widest point. Made of flexible plastic, the device was twisted to expand the gap opening, which allowed it to be fitted in the calf's nares where it hung freely without piercing the nasal septum. The device prevented nursing by covering the calf's mouth as the calf extended its neck and head toward the udder. Calves were still able to consume hay and silage and drink water while wearing the device. Abruptly weaned calves were allowed to nurse right up to the point of being physically separated from their dam.

The average age of calves on the day of separation was similar for both treatment groups (mean ± SD; two-stage=31.1 ± 2.7 and abrupt weaning=30.1 ± 3.9 d). However, because two-stage calves wore the antisucking device for 4 d they in fact nursed for fewer days than abruptly weaned calves.

On their assigned day of separation, calves were removed from the free-stall housing area during the morning milking period, while the cows were away. After separation, calves were housed individually in pens (1.5 × 1.2 m) located in a separate barn out of visual or auditory range of their dams. The cows remained in the same free stall area for a few days following separation. For the purposes of recording behaviour calves were kept in the individual pens described, but in a room away from other calves. Thereafter they were kept in an adjoining room, still in individual pens, but with calves in adjacent pens.

Given that calves in this study were born over a period of weeks, their respective dates for separation from the dam were also staggered by the same time gaps. Accordingly the number of animals present in the free-stall housing changed regularly.

7.3.3 Behaviour observations

A preliminary period of continuous 24-h observation was used to determine time periods when the animals were most active. Accordingly, an observation schedule was set to record behaviour for 7 h/d. All observations throughout this experiment followed the same time schedule. Animals were observed from 0500-0700 h (2 h), 1 h following morning milking, from 1400-1500 h (1 h), 1 h following afternoon milking and then from 1900-2100 h (2 h). All behaviour was recorded by direct observation except for the behaviour of calves after separation, which was documented from video recordings.

The experimental timeline for this study, outlining days that cows and calves were observed, is presented in Figure 7.1. Baseline information was recorded about the behaviour of every cow-calf pair on the last day that they were able to nurse; the day before calves were fitted with the antisucking device (two-stage pairs) or the day before separation (abruptly weaned pairs). To gauge the effects of preventing two-stage pairs from nursing they were observed for 7 h during the first 24-h period the calves were wearing the antisucking device and then again for 7 h during the fourth 24-h period they were prevented from nursing. All cows and calves were also observed for 7 h during the first 24-h after separation, following the schedule outlined previously. Cows and calves

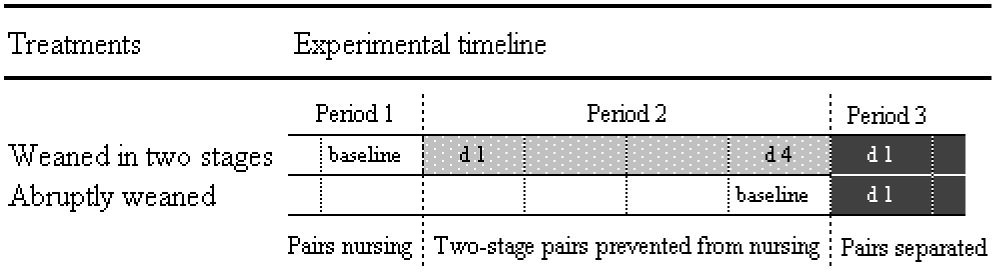


Figure 7.1. Treatments and the experimental timeline for the present study, illustrating the days when cows and calves were nursing □, days when two-stage cows and calves were together, but prevented from nursing ▩, and days when cows and calves from all treatment groups were apart ■. Behaviour of all cows and calves was observed on one baseline day (last day of nursing) and on d 1 after separation (Period 3). Additionally, cows and calves weaned in two stages were observed on the first day (d 1) and fourth day (d 4) that nursing was prevented.

were numbered with livestock paint to facilitate individual identification in the free-stall area.

The general activity of each animal was recorded at 10-min intervals, by instantaneous scan sampling. Observers recorded body posture (lying, standing or walking) and whether animals were eating, ruminating, drinking water, engaged in allogrooming between a cow and calf, or nursing. Behaviour categories were not all mutually exclusive. Allogrooming and nursing were scan sampled instantaneously every 60 s throughout the observation periods. Whether nursing and allogrooming occurred between dam-offspring pairs was noted as were instances when calves were in the calf-only area. When calves being weaned in two stages were prevented from nursing, their attempts to nurse were recorded by the same method used to record nursing (instantaneous scan sampling every 60 s). A nursing attempt was defined as the calf having its head or nose in contact with the cow's udder.

After being separated from their dam some calves made attempts to get out of the pen where they had been isolated for video recording. Pens had solid sides with a small keyhole opening on each wall for calves to access water and calf starter, which were hung outside the pen. Some calves were observed charging the opening and pushing against the wall with their shoulders once their head was through. This was clearly visible in video recordings and calves swung their tail from side to side as they pushed against the pen walls, sometimes physically moving the pen, which was not secured to the floor.

Given the natural distribution of calf birth dates only a few pairs were being observed on any given day. Data was collected by one person at a time and the majority of data over the course of the experiment was collected by two observers.

7.3.4 Statistical Analysis

Data were expressed as the rate of vocalizing (calls/h), and the percentage of observations where animals were observed performing each behaviour variable recorded.

Treatment effects were analyzed during the baseline period when pairs from both groups were nursing (Period 1) and after cows and calves were separated (Period 3). To gauge the response of two-stage cows and their calves when nursing (Period 2) was prevented day effects were examined, comparing behaviour on the baseline observation day to the first and fourth day that nursing was deprived.

All data were analyzed using a generalized estimating equations (GEE) method. Data were analyzed using a statistical computer software program (SAS v.8.2 for Windows (PROC GENMOD); SAS Institute, Cary, North Carolina, USA). Model specifications included a normal distribution, identity link function, and an AR(1) correlation structure. Where necessary the repeated statement subject was equal to animal identification, Variables remaining in the final multivariable model at $P<0.05$, based on the robust empirical standard errors produced by the GEE analysis, were considered statistically significant.

Data from 2 pairs (both the cows and the calves) assigned to two-stage weaning were discarded from the analyses of Periods 2 and 3 for not having received the full-intended treatment. Both pairs were observed nursing during the period that calves were wearing the antisucking device. This study was designed to investigate the effects of two specific weaning treatments, not the efficacy of the antisucking devices used. In every other way these pairs were handled the same as the other pairs in this study.

7.4 RESULTS

7.4.1 Period 1 (baseline)

Cows behaved similarly when they were nursing their calves, regardless of what treatment group they had been assigned to. Calves in the abrupt weaning treatment group spent a greater percentage of their time in the ‘calf-only’ area (65.7 vs. 42.9%, $P<0.05$) and calves assigned to two-stage weaning treatment tended to have a higher percentage of observations where they were drinking (3.3 vs. 0.6%, $P=0.10$).

7.4.2 Period 2 (Stage 1)

The behaviour of two-stage cows was not significantly affected by preventing them from nursing. Their behaviour on the baseline day when they were nursing was similar to their behaviour during the first and fourth 24-h periods after nursing had been deprived.

The effect of preventing nursing on the behaviour of calves is presented in Table 7.1. On the first day calves were fitted with antisucking devices, they spent 12.1% of observations attempting to nurse, which was significantly more time than they had spent nursing just the previous day (3.7%, $P<0.01$). The rate of vocalizing by calves was also significantly higher when nursing was prevented compared to baseline levels (6.7 calls/h vs. 0.7 calls/h, $P<0.05$). Four days after nursing was prevented the rate of vocalizing by calves was still significantly higher than baseline (4.1 vs. 0.7 calls/h, $P<0.05$) but similar to their rate of vocalizing on the first day nursing was prevented. Between the first and the fourth day without nursing, the percentage of time two-stage calves spent eating increased from 2.8% to 12.9% ($P<0.001$) as did the time they spent ruminating (12.6% vs. 28.3%, $P<0.01$). Time spent lying decreased over during the period nursing was prevented ($P<0.01$) time spent standing tended to increase compared to baseline (31.9 vs. 23.5% of observations, $P=0.06$).

Table 7.1. Mean (± SE) percentage of time each behaviour variable was observed for two-stage calves comparing days from Period 1 (baseline) and Period 2 (when calves were prevented from nursing). Means on the same line with different letters differ by at least $P<0.05$[1]. Observations of nursing and grooming were recorded every 60 s while all others were recorded every 10 min during 7 h of observation during a 24-h period.

Behaviour (units)	CALVES WEANED IN TWO STAGES					
	Period 1 (baseline)		Period 2 (prevented from nursing)			
			d 1		d 4	
Vocalizing (calls/h)	0.7 ± 0.6	a	6.7 ± 3.7	b	4.1 ± 1.2	b
Walking (% time)	4.2 ± 2.0	a b	1.2 ± 0.8	a	1.9 ± 0.7	b
Lying (% time)	59.4 ± 9.6	a b	60.3 ± 5.6	a	47.1 ± 5.6	b
Standing (% time)	23.5 ± 5.3	a	25.0 ± 4.1	a b	31.9 ± 3.8	b
Eating (% time)	6.7 ± 5.7	a b	2.8 ± 0.7	a	12.9 ± 3.2	b
Ruminating (% time)	14.5 ± 4.3	a b	12.6 ± 2.0	a	28.3 ± 6.0	b
Drinking (% time)	3.3 ± 1.7		2.0 ± 1.0		3.1 ± 0.5	
Nursing (% time)[1]	1.4 ± 0.3	a	3.3 ± 1.0	b	2.1 ± 1.0	a b
Being groomed (% time)[1]	0.6 ± 0.3		0.7 ± 0.4		1.0 ± 0.4	
Calf-only area (% time)	42.9 ± 11.8		42.1 ± 7.5		49.7 ± 14.2	

Means (±SEM) on the same line with different letters differ by at least $P<0.05$

7.4.3 Period 3 (Stage 2: following separation)

Behavioural responses of calves according to weaning treatments is depicted in Figure 7.2.

After separation abruptly weaned cows vocalized at a rate approximately double the calling rate of cows weaned in two stages (147. 1 vs. 74.6 calls/h, $P<0.05$). Abruptly weaned cows also spent less time ruminating (15.2 vs. 21.6% of observations, $P<0.01$).

The rate of vocalizing by calves after separation followed the same pattern as their dams. Calves weaned in two stages vocalized less than abruptly weaned calves (2.2 vs. 51.8 calls/h, $P<0.001$). In fact the calling rate by two-stage calves was not statistically different than their rate of vocalizing during baseline observations (2.2 vs. 0.7 calls/h). Abruptly weaned calves spent more time moving around in their pen after they were separated from their dam, compared with calves weaned in two stages (11.9 vs. 0.8% of observations, $P<0.001$) while the two-stage calves spent a greater proportion of their time lying (77.8 vs. 65.0% of observations, $P<0.001$). Two-stage calves also spent more time ruminating after separation (26.0% of observations) compared with calves weaned abruptly (13.1%). However, ruminating by abruptly weaned calves after separation was not different than the time they spent ruminating during baseline observations. During the seven hours observed following separation, abruptly weaned calves made on average (mean ±SE) 14.9±2.5 attempts to jump out of the pen they were being kept in, while no two-stage calf was observed making any similar attempts ($P<0.001$).

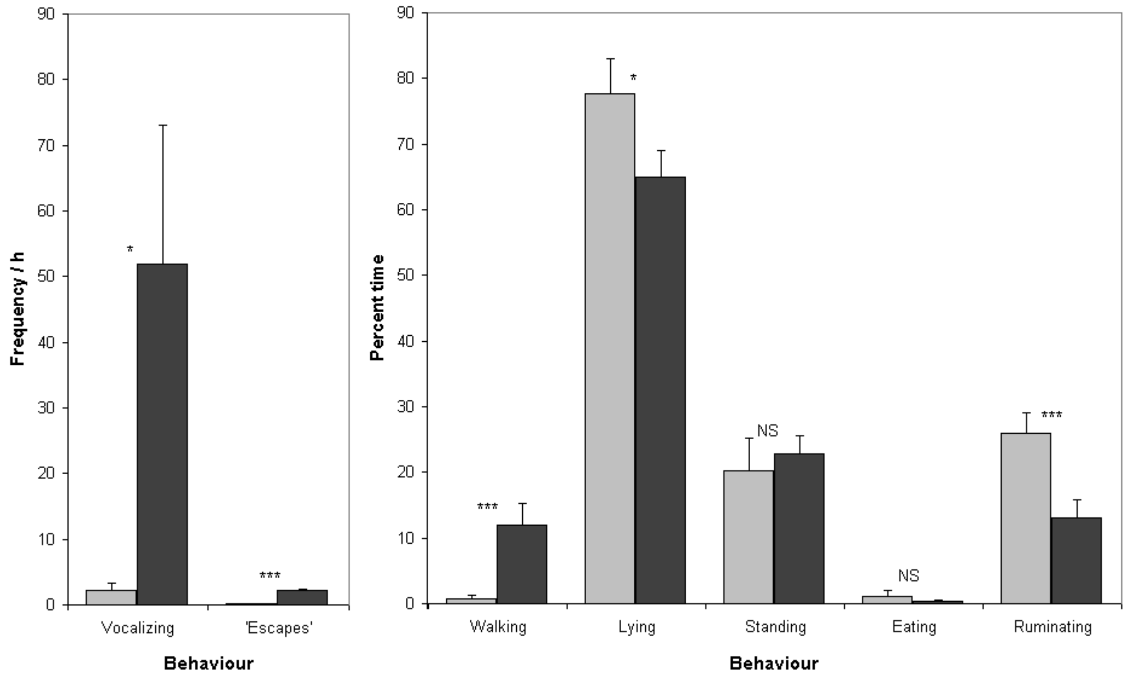


Figure 7.2. A comparison of the behavioural responses of calves to separation from their dam when weaned in two stages ☐, or weaned abruptly ■. Data represent the mean (±SE) vocalizing[1] and the 'escape' behaviour are expressed in frequency / h while all other behaviour variables are shown as their occurrence expressed as a percentage of the total time observed. Treatment means showing * differ by P<0.05; *** by P<0.001;

[1]Rates of vocalizing are estimates calculated based on the number of calls recorded during 2-min sampling intervals, every 10 min.

7.5 DISCUSSION

In this study, preventing nursing between cows and calves prior to their separation resulted in fewer behavioural signs of distress than weaning them abruptly by separation. The present results support previous findings, but also expand our understanding about the broad applicability of principles that underlie the effectiveness of this weaning method.

Weaning in this study was imposed on calves at 32 days of age that had been nursing their dams *ad libitum* since birth. Given the fact dams in this study were of dairy breeding it is assumed that milk supply was not a limiting factor for the offspring at the time of weaning and that calves were receiving a majority of their nutrition from their dam's milk. Thus, the present results show the two-stage procedure is similarly effective at reducing the distress of weaning for calves regardless of their state of nutritional dependence on the dam, suggesting it may indeed mimic the natural weaning process.

Interestingly, two-stage cows in this study showed virtually no response when nursing was deprived. In previous studies, beef cows around 205 d into lactation and of presumably lower milk yield did show a slight, though detectable behavioural response when nursing was prevented. The increased rate of vocalizing by beef cows could serve to stimulate nursing by the calf. In the present study dams were being milked twice daily throughout the experiment. Increasing intramammary pressure is one possible proximate cause behind the response of beef cows when nursing was prevented. However, this result may also reflect differences in the maternal behaviour of beef and dairy cattle. Maternal behaviour has not typically been an important selection criteria for dairy cattle.

Two-stage calves in this study increased their rate of vocalizing when they were prevented from nursing, the same behavioural response seen in previous experiments (Chapter 3). These young calves vocalized at a higher rate (5.4 calls/h) than those reported for older beef calves (1.1 calls/h; Chapter 3). These differences might reflect differences in their level of need for their dam's milk. The rate of vocalizing by piglets isolated from the sow after varying periods without nursing has been shown to provide reliable information about their need for the sow's resources (Weary and Fraser, 1995; Weary et al., 1997). It is possible the differences in calling rates between studies may be due to methodological differences as in the present study calves were only observed during 7 h representing their most active periods of the day.

The elevated rate of vocalizing by calves in the present study was sustained for and still significantly higher than baseline levels, even four days after nursing had been prevented. In comparison, calling by calves during Stage 1 in previous trials was generally only elevated on the second day without nursing and did not persist. Continued calling by calves in the present study may, again, reflect an increased level of need on their part in terms of their nutritional dependence on the dam's milk.

A previously unreported behaviour observed following separation in the present study was that several calves from the abrupt weaning treatment made what appeared to be attempts to break out of the pen where they were being kept during their first 24 h after separation. As this method of isolated housing has not been employed in the other studies it is unknown whether this is a response to separation from the dam, to isolation from other animals per se, or whether it is more specifically a reflection of the motivation of calves of this age to reunite with their dam. However, it is clear that weaning treatment had an effect on this behaviour as calves weaned in two stages were not observed attempting to jump through the openings in the pen wall despite the fact all calves were placed into the same isolation pen after separation. It is not known whether calves might also show this behaviour if weaned abruptly at a much older age (e.g., 205 d).

The fact that two-stage weaning caused fewer behavioural signs of distress for calves, even calves highly dependent on the dam's milk, could be considered evidence that the technique somewhat mimics the natural weaning process in that natural weaning occurs when milk is no longer available and not when the young reach a specific age.

8.0 SUMMARY AND CONCLUSIONS

8.1 Summary

The general objective of this thesis was to advance our understanding of certain factors that affect the response of beef cows and calves to the practice of artificial weaning. This series of experiments began with a goal of isolating the effects of two features that are inevitably confounded in the traditional weaning process, namely, the effects of terminating nursing and the effects of separating dams and their offspring. These two effects were successfully disentangled by the development of a novel two-stage weaning model. The model involved fitting calves with an antisucking device to prevent nursing for a period, prior to the physical separation of cows and calves. Based on behavioural responses neither of these two factors emerged as having clearly more important effects on the reaction of cattle to artificial weaning. Undoubtedly, the most striking result was the reduced overall behavioural response following separation that resulted from weaning by the two-stage method compared to traditional abrupt weaning. The initial study also documented that behavioural responses of the dams were greater than the responses of their calves.

Triver's theory of parent-offspring conflict would predict that dams should consent to weaning more easily than their calves in order to sustain reserves for investing in future offspring. By contrast, Triver's theory would predict that calves should more vehemently protest weaning as they have everything to lose and seemingly nothing to gain as an end result of weaning. However, based on measures of behaviour, results from the present studies suggest cows objected more to weaning than their offspring. Regardless of weaning treatment, cows responded by vocalizing almost twice as much as the calves. One interpretation of this finding could be that cows have a stronger attachment to their calves than calves have to their dams. When prevented from nursing by the antisucking device calves' nursing attempts did not seem to persist for very long, and their response to subsequent separation was negligible. From an evolutionary perspective it would seem that the dam does have more to lose in terms of negative effects on both her exclusive and inclusive fitness. Thus, it is conceivable that dams could have evolved a stronger attachment to their offspring to motivate the dam to be more attentive to the whereabouts and well-being of their offspring, than vice versa. By comparison, the cost to the offspring of losing the dam, or being separated from the dam would have a less significant effect with respect to fitness and thus calves may be less attached to their dam and view her primary function as serving first and foremost as a source of source of nutrition. This might explain the apparent ease with which two-stage calves appeared to relinquish leaving their dam once nursing had been terminated.

A second study examined whether preventing nursing between pairs for different durations would affect their behavioural response to subsequent separation. The underlying assumption was that spending a greater amount of time together without nursing would allow for a more complete dissolution of the dam-offspring bond and further diminish their response to separation. The results did not provide evidence that preventing nursing for 8 d changed the response to separation compared with preventing nursing for 4 d. However, taking into account the modest sample size from this study, there was some tendency for dams to respond less to separation when nursing was prevented for the longer period. Whether the degree of attachment differs for the dam and the offspring is an interesting question worthy of further investigation. An answer to this question could provide useful insight into the possible differences observed between cows and calves in their response to varying periods of milk deprivation, prior to separation.

Findings on the benefits of two-stage weaning fostered a growing interest from cattle producers in using this weaning technique. Requests for information about the logistics of implementing this management practice in the field supported the need for an on-farm trial. Typically calves given pre-weaning vaccinations are processed, for this purpose, two weeks prior to separating cows and calves. Thus a study was set-up to examine the practical benefits of fitting calves with antisucking devices two weeks prior to separation. In that study, conducted at Montana State University, calves wearing antisucking devices for 2 weeks weighed less at the point of separation than controls, and less than calves that wore the devices for 3 days. This result pointed to the potential importance of providing calves with supplemental nutrition when milk intake is prevented, especially in situations of limited grazing. In turn this study suggested the need for a possible fine-tuning of other weaning management activities to optimize the application of two-stage weaning. Regarding overall productivity, calves weaned in two stages and prevented from nursing for 3 d gained more weight during the 52 d following separation than abruptly weaned calves, and they also gained more weight than two-stage calves prevented from nursing for 14 d before separation.

During the Montana study, observations of cows and calves at pasture prior to separation confirmed that preventing nursing initially causes dams and their offspring to spend their time in closer proximity to one another compared with nursing pairs and pairs that had been prevented from nursing for 2 weeks. This finding could call into question the usefulness of spatial relations between dams and their offspring as a measure of the strength of their bond.

Guyot (1998) has described additional criteria necessary to determine attachment beyond seeking and maintaining proximity, including demonstrating a choice preference for the individual subject of attachment, a noticeable response to brief separations as well as extended separations, as well as a distinguishable response to reunion with the presumed figure of attachment.

The results of this study provided further evidence that, at least for calves, preventing nursing for longer (14 d) does not noticeably affect the behavioural response to separation compared to preventing nursing for a short period (3 d). Unfortunately, the behaviour of cows could not be examined in this study. The behaviour results suggest that, for calves, the benefits of weaning in two stages are gained at some point during the first 72 h that nursing is prevented. This finding is remarkable because it has been theorized that the negative response to artificial weaning is due to a strong and long-lasting bond between dams and their offspring. However, the fact calves could be separated from their dams with relative quiescence within a few days of nursing being prevented suggests that calves may not be strongly bonded to their dams once the supply of milk is terminated.

Fenceline weaning is considered to be an improved alternative weaning strategy. However, in comparison, two-stage weaning was shown to have less of a negative impact on the behaviour of calves than weaning by fenceline contact. The differences were significant in spite of minimal animal numbers. This comparison is worthy of more thorough examination to further document the nature of differences that result from imposing these two weaning treatments. Also, it would be logical to examine the benefits of combining these weaning methods by preventing nursing prior to separating pairs and then by permitting fenceline contact between dams and offspring after separation. Ultimately this may offer the best weaning method to date, but from a scientific point of view this blended weaning protocol may also provide a unique opportunity to see how pairs weaned in two-stages interact across the fence compared to pairs weaned by fenceline alone.

The final study in this thesis was more fundamental in nature, and returned to the importance of milk and maternal contact in the response of suckled cattle to weaning. The merit of two-stage weaning was tested on very young calves (35 days of age) much younger and far more dependent on their dam's milk than calves in any of the previous studies. The results of the trial further demonstrated the robustness of the principles underlying two-stage weaning, as a model that simulates certain important features of the natural weaning process. This final study showed that the beneficial effects of weaning in two stages is not limited to older calves that are close to being nutritionally independent and that even very young dependent calves benefit greatly from the protocol of being allowed contact with their dam when access to milk is prevented.

8.2 Conclusions

This thesis has documented that precluding nursing while pairs are still together has very negligible effects on their behaviour except for a relatively modest rise in vocalizing. Interestingly this was true even for very young calves (35 d of age) that were almost completely dependent on their dam's milk as a source of sustenance. In the present experiments all vocal sounds were lumped together, however, the acoustic characteristics of vocalizations when nursing was prevented appeared quite distinct from those heard following separation and those produced by abruptly weaned cattle. Calls given following separation were long and loud calls whereas calls during the period when two-stage pairs were prevented from nursing were often short and quite low, somewhat reminiscent of those vocalizations that occur soon after calving. Differentiation of the vocal qualities would have added an appealing dimension to these studies as vocal behaviour has been discussed as a possible indicator of the emotional state of individual animals, and their welfare.

It is difficult to draw clear conclusions about the relative effects that separating dams and offspring had on their behaviour. In every study in this series, cattle weaned in two stages were exposed to the potential influence of the stark responses of abruptly weaned control counterparts. While treatment differences could have operated in the opposite direction, with two-stage animals perhaps imparting some calming influence any effects of one treatment on the other did not obscure main effects between abrupt and two-stage treatments. Regardless, the question of comparing responses to the prevention of nursing relative to separation remains confounded in these studies. Also, order effects limit conclusive interpretations based on the present results. It may be possible to familiarize dam-reared calves with taking milk from a bottle or pail, which would permit separation of calves from their dam before milk feeding is prevented.

A very important conclusion to be drawn from these research results is that the most widespread method of weaning cattle, by the abrupt separation of pairs, in fact aggravates the level of distress that the animals experience. Separating dams and their offspring to terminate nursing has synergistic effects, causing a behavioural response that is greater than the collective responses caused when these two factors are imposed independently. Also, the age of the offspring relative to the natural weaning age appears to be of less importance than the actual details of the way in which artificially weaning is imposed.

This thesis work has uncovered a novel method for weaning cattle in two stages that can be practically applied. This technique produced consistent results across all of the trials in terms of substantially reducing the behavioural signs of distress following separation. Allowing cows and calves the opportunity to fully engage in nursing attempts is an element that may be responsible for the reduced behavioural response observed when cattle are weaned in two stages. As a comparable method, fenceline contact may also offer pairs the possibility for some interaction, however, the most obvious difference is that the use of nose-flaps with two-stage weaning allows for the pairs to engage in complete nursing attempts. It may also be that other specific physical interactions such as grooming, or other more general aspects of physical contact also contribute to the quelled response although these may also be permissible by fenceline contact.

In some ways, the work in this thesis has just begun to explore the benefits and the mode of action of two-stage weaning as a possible alternative to abrupt weaning. Further consideration should be given to proving the extent to which physiological signs of distress are affected by weaning in two stages. The basic question on the order in which the nursing or social contact are removed and whether this is crucial to the outcome and effectiveness of two-stage weaning has not yet been determined. And the ultimate question arising from the present results is whether the beneficial differences in behaviour observed have practical implications that significantly affect morbidity rates among calves after two-stage weaning.

Other practical considerations also need to be explored further to help realize the possible benefits of weaning cattle in two stages, including the provision of supplemental feed for calves when nursing is prevented, especially if that phase is prolonged. Also the same effect of terminating nursing may be achievable by other means (e.g., perhaps methods affecting the flavour of the dam's milk to stop the calf suckling; perhaps methods to facilitate the end of milk production via endocrine pathways, etc.).

From a theoretical perspective of trying to understand dam-offspring bonds and the nature of their interactions, the present technique offers a robust experimental model that could be used to pursue a wide array of research for various species. Inevitably the question arises as to whether similar beneficial effects could be achieved by applying the same two-stage method to wean other farm animal species.

Observations regarding the spatial relationship between dams and offspring prevented from nursing raise questions about using the physical proximity of dams and offspring to assess the strength of their bond. Counterintuitive to prevailing theories, cows and calves observed spending their time in close physical proximity responded less to separation than pairs observed spending their time further apart. Maintenance of physical proximity is only one possible measure that a bond exists, though it is often the primary indicator used in research on bonds and attachment.

The fact that preventing nursing for even 3 d has such remarkable effects on reducing the response of cows and calves to permanent separation would appear to challenge the notion that bonds between the dams and offspring are considered to be long-lasting, even after artificial weaning. One interpretation of the present results could be that for calves, the bond may be almost entirely based on the dam as a source of milk. However, tests specifically directed at assessing the bonds between cows and calves would need to be aligned with the present model. The results also beg the question of exactly when, during the first 3 days without nursing, the benefits of two-stage weaning are achieved for calves. It is also unknown whether this is a direct effect of time or it if coincides more with observable behaviour changes such as following the period the sharpest decline in the frequency or duration of nursing attempts.

Much more detailed observations are certainly warranted of stage one period, when nursing is prevented, as the present studies only grossly documented general activity. Observations should be targeted at closely documenting the interactions between dams and offspring and trying to understand how they might relate to individual differences in terms of their response to separation. As previously stated, it may be valuable to perform further experimental manipulations during this period including specific tests to assess the motivational strength of dams and offspring to be together. Another possible intervention might involve manipulations to test the role of specific physiological parameters believed to influence the nature of the dam-offspring interaction (e.g., oxytocin).

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APPENDIX A: INTEREST IN TWO-STAGE WEANING BY THE CATTLE INDUSTRY

The beneficial effects of two-stage weaning on the behaviour of cows and calves were so remarkable and so easily achieved that a concerted effort has been made to communicate these practical research findings to the cattle industry. The first farm-press article on this topic appeared in BEEF Magazine in November 2001. Since that time, two-stage weaning has been the focus of other articles and farm media television reports, both in English and in French. Interest in trying the two-stage method of weaning cattle has spread. Between June 2002 and June 2006 over 750 separate inquiries for information were received to seek advice about weaning cattle in two stages, and asking for assistance in locating the antisucking devices I used in my studies. A number of cattle producers have now at least tried this weaning method. During the same period of June 2002 and June 2006 one company alone has sold 111,100 antisucking devices to producers specifically inquiring about using them for two-stage weaning.

Select farm press articles on two-stage weaning:

The Weaning Two-Step, by Joseph M. Stookey and Derek B. Haley
BEEF Magazine, November 2001, article starts on page 30

2-Step Weaning, by Derek B. Haley
Canadian CATTLEMEN Magazine, June / July 2002, article starts on page 26

More on two-step weaning, by Derek B. Haley, Joseph M. Stookey and Derek W. Bailey
BEEF Magazine, September 2003, article starts on page 54